Effects of predation on the demography and genetics of a facultatively parthenogenetic *Daphnia* population

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Leif K. Hembre, 243 words

ABSTRACT

This research examines the demographics and genetics of a facultatively parthenogenetic *Daphnia* population, and provides novel insight into how an ecological interaction (predation) affects 1) the diversity of a spatially structured clonal population and 2) whether clonal selection or recruitment from resting eggs will control clonal diversity.

This research also demonstrates the power of high frequency sonar for the rapid assessment of zooplankton spatial distribution, and for a comprehensive evaluation of population size. Sonar data were used to analyze the predator-prey relationship between *Daphnia* and rainbow trout.

A demographic analysis of these data showed that trout predation is a significant source of mortality to *Daphnia* during the winter, when the reproductive rate of *Daphnia* is low. Predation over winter depresses the *Daphnia* population and prevents it from growing explosively in the spring, thus resulting in low grazing rates, high phytoplankton abundance, and relatively turbid water. In contrast, when *Daphnia* are free from trout predation over the winter, the population fortifies and has a high reproductive potential by spring. The high reproductive rate of the *Daphnia* population enables it to grow in spite of heavy predation by spring-stocked trout. Grazing by the large spring *Daphnia* populations appeared to cause extremely transparent water in the late spring and early summer of these years. These findings should be relevant to lake and fisheries managers, because the results reveal a management strategy that optimizes seemingly contrary objectives - i.e., a productive planktivore sport fishery and clear water.

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PREFACE

Overview

The ability of *Daphnia* to switch between asexual (parthenogenesis) and sexual reproduction has important ecological and evolutionary consequences. Ecologically, this reproductive flexibility enables individuals to rapidly reproduce when resources are abundant. *Daphnia* populations, therefore, can exhibit explosive growth and during which they may graze phytoplankton to very low concentrations, resulting in high water transparency. When conditions worsen (e.g., scarce resources, high predation) individuals may form a diapausing egg capsule (an ephippium) containing haploid eggs and reproduce sexually. Ephippia serve as important dispersal vectors through space (i.e., they may be transported among water bodies) and time (i.e., they may emerge sediments decades after they are deposited). Evolution in facultatively parthenogenetic *Daphnia* populations depends on the interaction between selection acting on clonal lineages in the active population, and immigration from ephippia.

This dissertation examines the demography and genetic structure of facultatively parthenogenetic *Daphnia pulicaria* population in a lake in northwestern Minnesota (Long Lake) that is annually stocked with a rainbow trout, a predator of *Daphnia*. High-frequency (192 kHz) sonar was used to determine the abundance and spatial distribution of *Daphnia* and trout. The first chapter describes how the sonar system was used to estimate *Daphnia* abundance, and examines the patchiness of the population from spring to fall of one year. Knowledge gained from the acoustic analyses of the *Daphnia* population is incorporated in the next two chapters. Chapter 2 explores how the timing of predation by trout affects the demography of the *Daphnia* population and the lake's water

clarity. The next chapter (Ch. 3) examines the ecological genetics of the *Daphnia* population, and investigates how patterns of genetic structure and diversity within and among years are affected by changing environmental (i.e., stratification) and ecological (i.e., trout predation) conditions. The final chapter (Ch. 4), which was inspired by my research on *D. pulicaria* and by new biotechnological advances that have enabled the cloning of mammals, expands the consideration of facultative asexuality beyond *Daphnia*.

Chapter 1: Using the inaudible to see the invisible: An acoustic analysis of a *Daphnia* population

The objective of this research was to develop methodology to quantitatively assess the abundance and spatial distribution of *Daphnia pulicaria* using sonar. Sonar is a valuable tool because it provides "real-time" information about the spatial distribution of zooplankton to guide sampling, and when used in conjunction with conventional sampling methods, is a powerful alternative to blind sampling.

Data from conventional samples taken while recording acoustic information were compared to the sonar data to calibrate the sonar information. Regression analyses revealed that the concentration of large *Daphnia* was the only significant predictor of mean volume backscattering (MVBS). This result is likely due to the fact that *D. pulicaria* is substantially larger than other zooplankters in the lake, and because MVBS is proportional to the sixth power of body radius. Given that MVBS was largely dependent on the concentration of large *Daphnia*, we used the data to calculate the target strength of a "*Daphnia* equivalent" which was determined to be -120 dB. We used this target

strength to calculate the total *Daphnia* population size on each sampling date in 1998. To our knowledge this is the first study to use sonar to calculate population sizes. We also discuss the use of acoustic analyses to investigate the forces that structure zooplankton patchiness.

Chapter 2: Dependence of *Daphnia* demography and water clarity on the timing of trout predation

Long Lake is a popular recreational lake, and is valued for both for the aesthetic appeal of its clear water, and because the rainbow trout provide a unique sport fishery. The challenge to the fisheries managers from the Minnesota Department of Natural Resources (MDNR) has been to provide a sport fishery, without sacrificing the lake's water clarity via food chain effects (i.e., increased trout → decreased *Daphnia* → increased algae → decreased water clarity).

Through coordinated efforts with fisheries managers from the MDNR, the stocking program was altered in 1998. Instead of stocking trout in the autumn, the fish were added to the lake soon after ice-out. This change allowed me to assess the importance of the timing of stocking to the demography of the *Daphnia* population, water clarity, and the survivorship of the trout.

The switch to spring stocking reduced *Daphnia* mortality during the winter and allowed a substantial number to survive. The large spring populations exploited the spring phytoplankton blooms and produced large broods. The reproductive rate of the spring populations was high enough to offset the mortality caused by the newly stocked trout. From a management perspective, this outcome was a virtual panacea. The *Daphnia*

population was large early in the open-water season and exerted strong grazing pressure on the algae, which resulted in significantly clearer water than in the previous two years when trout were stocked in the autumn. Also, the abundant *Daphnia* provided trout with an abundant food supply early in the summer and trout survival through the summer was improved.

Chapter 3: Controls on annual and inter-annual patterns of genetic structure and diversity of a *Daphnia* population subjected to trout predation

This research builds on the understanding of the predator-prey relationship between trout and *Daphnia* and examines how the alteration of the predation schedule affected the clonal composition and diversity of the *Daphnia* population.

The emergence of sexually-produced individuals was detected (agreement with Hardy-Weinberg equilibrium expectations) in late spring-early summer of years after fall stocking, when the active *Daphnia* population was at low density. In spring stocking years, when the *Daphnia* population was large, ephippial emergence was not detected. This finding underscores the importance of ecological conditions on the diversity of facultatively parthenogenetic populations. It is likely that when the resident, clonally-reproducing population is large, emergence is undetectable or insignificant. These results also suggest that if the spring stocking strategy continues year after year, parthenogenetic reproduction will be promoted and the clonal diversity of the population will inevitably decline.

Chapter 4: Evolutionary and behavioral consequences of cloning

This chapter incorporates understanding gained from my research on *Daphnia*, but more broadly explores evolutionary consequences for populations that switch between sexual and asexual reproduction. Specifically, I compare how populations that switch between asexual and sexual reproduction differ in their exploration of adaptive landscapes (*sensu* Wright 1931) from obligately sexual populations.

Chapter 1: Using the Inaudible to See the Invisible: An Acoustic Analysis of a *Daphnia* Population

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Abstract

To accurately estimate the size of zooplankton populations, the location and extent of patches must be known. We used high-frequency (192 kHz) single-beam sonar to map zooplankton aggregations in a Minnesota lake during spring, summer, and autumn. Samples collected with a plankton net from aggregations of sound-scatterers revealed that most backscattered sound was attributable to *Daphnia pulicaria*. This large cladoceran is a Rayleigh scatterer of 192 kHz sound. With a mean body length of 1.6 mm, it is in a size-range where backscattering is proportional to the 6^{th} power of body length. Because Rayleigh scattering is so dependent on size, other planktonic crustaceans in this lake, which also are Rayleigh scatterers but are less than 1/2 the size of *D. pulicaria*, have little effect on backscattered sound. Most of the variance (63%) of acoustic backscattering was attributable to *D. pulicaria* with a mean target strength of -120 dB.

Acoustic transects, which yielded ~ 2×10^6 samples per km, revealed aggregations of sound-scatterers near the lake surface during daylight in spring (April). After the lake stratified (May), the daytime aggregation was deeper, below the metalimnetic maxima of oxygen and chlorophyll, in water where temperature was < 9 °C, and oxygen was > 1 mg liter^{-1}. The deep layer of sound-scatterers was compressed during mid-summer and early autumn as the thermocline migrated downward and oxygen concentrations disappeared near the lake bottom. The thickness of the deep-water

aggregation decreased from about 15 m in May to < 2 m in late October. The daytime aggregation migrated upward and dispersed toward the lake surface after sunset.

The total quantity (+/- s.e.) of sound-scatterers in the lake, estimated in terms of a quantity of *Daphnia* equivalent to that of sound-scatterers with target strength of -120 dB, ranged from a maximum of 4.81×10^{11} (+/- 8.81×10^{10}) in late May to a minimum of only 1.77×10^{9} (+/- 6.28×10^{8}) in October.

Introduction

Analyses of lake zooplankton have been impeded by the patchy spatial distributions of plankton populations and the low resolution of conventional sampling methods. Zooplankton concentrations can vary by a factor of 1000 within distances of meters horizontally or vertically, and the sampling resolution of conventional plankton nets and hoses usually is too coarse to precisely identify the spatial limits of aggregations (Coyle 2000). Variation in population density estimates due to sampling often cannot be distinguished from real changes of population density, and the effects of biological processes are difficult to distinguish from those of advective transport (Megard et al. 1997).

Acoustic and optical plankton samplers developed during recent decades are major advances. They have very high sampling rates and spatial resolution, comparable to modern instruments used to measure environmental variables. Large numbers of plankton samples can be obtained rapidly from large geographic areas. The new technology dramatically improves our ability to locate and describe aggregations and to identify how aggregations are related to physical variables (e.g., Ross et al. 1996; Megard et al. 1997; Zhou et al. 2001).

High-frequency sonar can detect sound-scatterers in the size-range of zooplankton. Sonar by itself cannot discern the biological identities of sound-scatterers in aggregations, but it can be used in conjunction with plankton nets to identify scatterers and gain other information that neither method can provide alone. A large number of acoustic samples can be acquired and displayed rapidly to delineate aggregations at high precision with respect to depth and geographic coordinates. Surveys along transects

therefore can detect and record major features of spatial distribution that are invisible with conventional sampling. Because sonar data can be displayed instantaneously, they also can identify sites and depth increments for more intensive sampling with plankton nets and other devices. Depths to be sampled with nets can be selected with respect to the locations of subsurface sound-scatterers discerned simultaneously with sonar. Zooplankton investigations are no longer constrained by limits imposed by conventional "blind sampling" with nets at localities and depths selected arbitrarily.

Here we use single-beam high-frequency sonar to investigate the properties of a population of a *Daphnia pulicaria*, a planktonic cladoceran that congregates in the metalimnion of a Minnesota lake (Long Lake) in the daytime during the summer. The metalimnetic propensities of *D. pulicaria* were first documented more than a century ago by E.A. Birge (Birge 1895) in his study of the plankton of Lake Mendota, Wisconsin, but it is notable that *D. pulicaria* migrates into surface water at night in Long Lake (Hembre 1996). With a body length up to 3 mm, *D. pulicaria* is larger than most other cladocerans. The properties of *D. pulicaria* populations are of special interest because they and other large-bodied cladocerans are significant ecologically and economically. They feed efficiently on phytoplankton and are often most abundant during a phase of "clear-water" that occurs in many lakes during late spring and early summer (e.g., Luecke et al. 1990; Wright and Shapiro 1990; Hembre 2002a). In Lake Washington, grazing by *D. pulicaria* on phytoplankton apparently had a larger effect on water transparency than was achieved by decreasing the influx of nutrients from the lake's watershed (Edmondson and Litt 1982; Megard 2000). *Daphnia pulicaria* (a member of the *D. pulex* group) is important for fisheries management in the lake we studied as in other lakes, because it is the

dominant food for rainbow trout (*Oncorhynchus mykiss*) and other cold-water salmonids, which also congregate in lake metalimnions during summer (e.g., Bevelhimer and Adams 1993; Geist et al. 1993; Wang et al.1996).

Previous studies (Ross et al. 1996; Hembre 1996) of the *D. pulicaria* population in Long Lake, used acoustic methods qualitatively to locate aggregations for sampling, and to provide information about the overall spatial distribution of the population. To date, few acoustic studies have analyzed freshwater zooplankton populations quantitatively (exceptions: (Rudstam et al. 1992; Melnik et al. 1993; Megard et al.1997; Gal et al.1999). The research presented here will show 1) how aggregations of sound scatterers vary spatially and temporally in Long Lake, 2) the relative contributions by *D. pulicaria* and smaller zooplankton to the total strength of backscattered sound, 3) the target strength *D. pulicaria* and 4) the implications of high-resolution acoustic sampling for zooplankton analyses in the future.

Methods

Study Site

Long Lake is a dimictic, oligo- to mesotrophic lake located in northwestern Minnesota (latitude 47 17' N, longitude 95 17' W). The lake has a single basin, is 2.4 km long, has an average width of 300 m, a surface area of 66.5 ha, and a volume of 7.63 x 10^6 m^3. The basin is symmetrical and steep-sided, relatively deep (maximum depth = 24 m, mean depth = 13 m), and has a small littoral zone (~ 15% of lake surface area). Its depth and simple morphometry make it ideal for acoustic analysis, and because interactions in the pelagic zone dominate the ecology of the lake, an understanding of the spatial distribution and abundance of *Daphnia* is especially relevant.

Instrument design and capabilities

We used a sonar system described earlier (Megard et al. 1997) to sample zooplankton in Long Lake at high resolution. The system consists of a Lowrance X-16 high-frequency (192 kHz) single-beam echosounder and a Loran-C navigation receiver connected to a portable computer. A narrow-beam transducer (4° half angle), directed vertically and suspended from the bow of the boat approximately ½ m below the lake surface, emitted approximately one acoustic pulse (100 μs duration) per second. An analog-digital converter in the computer digitizes voltage variation due to sound back-scattered by zooplankton, fish and other particles in 2000 50 μs increments (bins), which correspond to depth increments of ~ 4 cm (28 acoustic samples m^{-1}). The system can collect a large number of samples (~ 2 x 10^6 km^{-1}) during an acoustic transect (boat speed ~ 5 km h^{-1}, mean water depth ~ 20 m).

The software (described in Megard et al. 1997) calculates the strength of backscattered sound from the digitized signal strength in terms of volume scattering strength, using the sonar equations (Urick 1983) to compensate for transmission losses due to beam-spreading as sound travels from the transducer to scatterers and returns to the transducer. Volume scattering strengths, calculated with reference to a standard tungsten-carbide sphere (Foote and MacLennan 1984), are displayed in the format of an echogram on the computer monitor and saved on the hard disk of the computer. Depths to be sampled with nets or other devices can be selected efficiently, with respect to the locations of sound-scatterers, because echograms are displayed instantaneously.

Most of acoustic backscattering in this lake is due to *D. pulicaria*, because it is larger and usually more abundant than other zooplankters. Planktonic crustaceans the size of *Daphnia* are Rayleigh scatterers of 192 kHz sound, with body lengths (0.2 - 3 mm) that are shorter than the wavelength (7.5 mm) at this frequency. Rayleigh scattering is proportional to the 6^{th} power of size (Clay and Medwin 1977; Holiday and Pieper 1980, 1995; Stanton 1989). *D. pulicaria*, with mean body length ~ 1.6 mm, is about twice as large as the second-largest zooplankter (usually calanoid copepods) in the lake (Table 1), so scattering by individual *Daphnia* is about $2^6 = 64$ times larger than that of the second-largest taxon.

Volume backscattering strength, expressed in terms of decibels, is

$$S_v = 10 \log s_v, \qquad (1)$$

where s_v, the volume backscattering coefficient, is the strength of backscattered sound at a distance of 1 m from an ensonified water volume. The volume backscattering coefficient in a water volume depends on the concentration of animals N (m^{-3}) and their backscattering cross section, σ_{bs},

$$\sigma_{bs} = s_v / N \qquad (2)$$

The backscattering cross section depends on the size of an acoustic target and often is expressed logarithmically in decibels as the target strength,

$$TS = 10 \log \sigma_{bs} \qquad (3)$$

The size of scatterers can be calculated in terms of their equivalent spherical radius from backscattering cross-section and wave number $k = 2\pi/\lambda = 838\ m^{-1}$ with

$$a = \{\sigma_{bs} / [k^4 \alpha^2]^{-1}\}^{1/6}, \qquad (4)$$

where $\alpha = 0.056$ is an acoustic contrast coefficient that depends on the relative density (g) and relative speed of sound (h) in a sound-scatterer (Table 1 in Stanton 1989).

Field operations

To obtain information about the distribution and abundance of zooplankton across the whole lake, we collected acoustic data while traveling at about 5 km h^{-1} along a transect of the lake's long axis from the southeast to the northwest end of the lake.

Acoustic data to be quantitatively compared with net sampling data were obtained while anchored in deep water (22-24 m). Sampling depths were selected by inspection of sound-scattering layers displayed on the computer (Fig. 1). We sampled discrete depth increments with a closing Wisconsin-style plankton net (27 cm diameter, 130 μm mesh size) within 15 minutes of recording acoustic data. Samples were preserved in the field with a chilled sucrose-formalin solution (Prepas 1978) and refrigerated until they were analyzed. Temperature and dissolved oxygen concentration were measured at 1-m intervals with a YSI model 58 dissolved oxygen meter.

Laboratory methods

For each sample, the number of animals in five 5-mL subsamples were counted in 6 categories: large *Daphnia pulicaria* (≥ 1.3 mm in length), small *Daphnia pulicaria* (< 1.3 mm in length), calanoid copepods, cyclopoid copepods, nauplii, *Diaphanosoma* and *Bosmina*. The 1.3 mm threshold for dividing *D. pulicaria* into 'large' and 'small' categories was chosen because it was the smallest size of an individual with a brood (Hembre 2002a). The body lengths of 15-25 individuals of each taxon were measured to the nearest 0.05 mm. Biomass of individuals was calculated from body length with regression equations (Malley et al. 1989).

A subset of the samples (n=32) was also analyzed for displacement volume (Postel et al. 2000). To determine the volume displaced by the organisms, a sample was first poured into a 25 mL graduated cylinder to measure its volume. The sample was then filtered using a glass fiber filter and gentle vacuum pressure (~ 250 mm Hg). The difference between the initial volume of the sample and the volume of the filtrate is the displacement volume.

Calibration of Acoustic Data

Target strengths of *Daphnia* (Eq. 3) were computed with data from thirteen dates between 11 October 1996 and 16 May 2000 (Table 1). These dates were selected because acoustic data were collected just prior (< 15 min) to net sampling. Mean volume backscattering strength (MVBS) in depth increments sampled with plankton nets was calculated from volume backscattering strengths from 50 pings, at a vertical resolution of ten acoustic samples per meter. Saturating signals from large targets (presumably fish) were omitted.

Results

Spatial Variation of backscattered sound

Backscattered sound can vary by a factor of 100 in Long Lake, as illustrated by an echogram in late May, 1998 (Fig. 2) for the long axis of the lake, a distance of 2.5 km. Mean volume backscattering strength (MVBS) varied between -80 and -60 dB. Three layers of sound-scatterers were detected. A layer of low backscattering between 5 and 8 m separated a layer of moderate scattering near the lake surface from a third layer in deep water. MVBS, typically near -70 dB in the surface layer and -80 dB in the intermediate layer, was less variable in the surface layers than in the deep layer, where it ranged from -60 dB in a dense eastern aggregation down to -80 dB near the western end of the lake (Fig. 3).

Temporal variation

The distribution of sound-scatterers changed substantially during a span of 6 months, as shown by long-axis echograms obtained during daylight on other dates in 1998 (Fig 4). Most sound scatterers were aggregated near the surface at the west end of the lake in April. The densest daytime aggregations in subsequent months were in deeper water, near the east end of the lake in May and June but near the west end in July and August. A number of curving echotraces indicate pathways of fluid flow and suggest sound-scatterers were transported by internal waves and non-periodic water movements. Sometimes layers were tilted by seiches.

The depth and thickness of the deep sound-scattering layer suggest that its location depended on environmental conditions and behavior of the sound-scatterers.

The deep layer was about 12 m thick in May, but only 2 m in October. It was compressed as the surface mixed layer (epilimnion) became thicker, the thermocline migrated downward, and dissolved oxygen disappeared from the deepest water. The top of the layer during daylight was related most closely to water temperature, and the bottom to oxygen concentration. Water temperature was 7-10 $^{\circ}$C and dissolved oxygen was > 5 mg liter $^{-1}$ above the layer. The bottom of the layer extended to the bottom of the lake in May and June, when the deepest water contained oxygen, but it moved upward in July, August and October as dissolved oxygen in deep water fell below 1.5 mg liter $^{-1}$ (Fig. 5).

Effect of zooplankton on backscattered sound

Both MVBS and mean zooplankton concentrations were computed for 49 depth increments in order to determine how zooplankton concentration affects backscattering. The depth increments, typically 2-3 m thick, were identified with sonar and sampled on 13 dates in spring, summer and autumn during 4 years (Table 1).

To determine what types of zooplankton significantly affected backscattering, the MVBS data from the sampling depths were regressed against net-sampled densities (10 Log_{10} (x+1) transformed) of the various types of zooplankton. The regression was highly significant ($p < 0.0001$) and explained 67% of the variance in MVBS (Table 2a). Large *Daphnia* was the only predictor variable with a significant p-value in the regression. When the large *Daphnia* variable was removed from the analysis, the regression was not significant ($p= 0.377$). A simple regression of MVBS on the density of large *Daphnia* (10 Log_{10} (x+1) transformed) was significant ($p < 0.0001$) and explained nearly as much of the variance (63 %) in MVBS as the multiple regression (Table 2b).

Volume backscattering was also regressed against the biomass of all the zooplankton taxa (Log_{10} (x+1) transformed). Fewer samples (n=39) were included in this analysis because the body lengths of animals in the samples from 1996-1997 were not determined. This regression was also highly significant ($p < 0.0001$) and explained a higher percentage of the variance ($R^2 = 0.79$) than did the regression on zooplankton concentration (Table 3). In this analysis, *Daphnia* were not separated into 'large' and 'small' categories. When *Daphnia* biomass was removed as a predictor variable from the analysis, the regression was no longer significant ($p = 0.176$).

MVBS increases as the concentration of large *Daphnia* increases (Fig. 6), but it is independent of smaller *Daphnia* (body length <1.3 mm) and other planktonic crustaceans, all smaller than large *Daphnia* (Table 2a, statistics). Another measure of abundance, total zooplankton volume (displacement), also covaries with *Daphnia* concentration (Fig. 6), but not with concentrations of smaller crustaceans and is an independent indication that the total volumes of other crustaceans usually were less than those of *Daphnia.*

Much of the variance of MVBS not attributable to *Daphnia* concentration (Fig. 6) probably is due to variation of the target strength of individuals, which depends strongly on body size. Target strength is proportional to the 6^{th} power of equivalent spherical radius (Eqs. 3 and 4). The mean target strength (-120 dB) corresponds to 0.28 mm equivalent spherical radius (esr). This is about 20% of the mean body length of *Daphnia* (1.6 mm) computed independently from measurements of individuals with an optical micrometer (Table 1). Almost all MVBS estimates in Fig. 6 are within limits computed for concentrations of animals with target strengths between -130 dB and -110 dB and esr

between 0.19 and 0.40 mm. The body volumes of individual *Daphnia*, computed for minimum, mean and maximum target strengths are 0.03, 0.09 and 0.27 mm^3 respectively, and thus vary by a factor of 10.

The total biomass of planktonic *Daphnia*, computed with empirical equations (Malley et al. 1989) from *Daphnia* concentration and body length, explains a larger proportion ($R^2 = 0.73$) of the variance of MVBS (Fig. 7) than does *Daphnia* abundance alone (Fig. 6), because total biomass depends on both abundance and size. *Daphnia* comprised most (60 - 90 %) of the zooplankton biomass during spring and summer, 1998, but only 20% in October (Fig. 8).

Most of the scattering of high-frequency sound in Long Lake is by *D. pulicaria* because *D. pulicaria* is bigger and more abundant than other planktonic sound-scatterers. The total quantity of scatterers in the lake therefore can be estimated in terms of *D. pulicaria* equivalents, where a *D. pulicaria* equivalent is a scatterer with a target strength of -120 dB. To transform measurements of backscattered sound to equivalent concentrations of *Daphnia*, the MVBS in depth increments where *Daphnia* occurred was divided by this target strength. The volumes of depth increments were then multiplied by numbers of *Daphnia* equivalents, and the sum of these products was taken as a measure of the total number of *Daphnia* in the lake. The total number of *Daphnia* equivalents was divided by the lake volume (7.63×10^6 m^3) to estimate the mean lake-wide concentration of *Daphnia* ($N\ m^{-3}$). In 1998, the number of *Daphnia* equivalents in the lake varied by more than two orders of magnitude from a high of 4.81×10^{11} *Daphnia* equivalents in May to a low of 1.77×10^9 in October (Table 4).

Discussion

The inherent patchiness of zooplankton populations has limited the ability of researchers to accurately determine population sizes because the location and spatial extent of these patches are largely invisible to conventional sampling methods. Here we have shown that acoustic sampling together with net sampling can provide the necessary detail to more comprehensively estimate population size.

There are several sources of variation between acoustic and net sampling estimates of zooplankton concentration. These include 1) variations in the scattering characteristics of individual animals (Chu et al. 1992), 2) scattering by non-focal species (Coyle 2000), and 3) net avoidance or inefficiency (Greene et al. 1989). Given these sources of variability, the relationship we found between MVBS and the concentration of large *Daphnia* from net samples was quite strong ($r^2 = 0.63$) and is within the range of relationships ($r^2 = 0.23$ - 0.93) found in other studies of freshwater zooplankton populations (Coyle 2000). The relationship was even stronger for the regression of MVBS on *Daphnia* biomass ($r^2 = 0.73$, Fig. 7), which incorporated information on *Daphnia* concentration and mean size.

The zooplankton community of Long Lake was amenable to this analysis because *D. pulicaria* dominates the biomass of the zooplankton for much of the year (Fig. 8) and is larger than the other zooplankton taxa (Table 1). Because backscattering intensity is proportional to the sixth power of the radius of a target (Clay and Medwin 1977; Megard et al. 1997), the sound backscattered by *D. pulicaria* obscured the signals sent by smaller, co-occurring taxa (Tables 2 and 3). Had our focal species been a smaller zooplankter the single frequency acoustic information we obtained would not have allowed for this kind of analysis.

Acoustic Estimation of Population Size

The dependence of MVBS on the concentration of large *Daphnia* enabled us calculate the target strength of *Daphnia* (Table 1). The target strength, together with MVBS data from long-axis transects of the lake (Fig. 4) allowed us to extrapolate the total size of the population (as *Daphnia* equivalents) in 1998 (Table 4). To our knowledge, this is the first study to use acoustic data to make this calculation. This method for calculating population size is relevant for ecological studies of pelagic food webs, and could be used by fisheries managers to aid decision-making regarding the timing and the level of fish stocking (Hembre 2002a).

Precision Sampling

Another major advantage of using the sonar system we employed is that the location and limits of aggregations were immediately discernable. Access to this "real time" data was especially useful for the study of habitat partitioning by clones of the Long Lake *D. pulicaria* population (Hembre 2002b). By precisely sampling aggregations at specific depths I was able to document the genetic differentiation of the population with respect to depth, and to identify clones with different habitat affinities. Had I sampled at fixed depth intervals, the samples collected would likely have been a mixture of patches that differed genetically, and my ability to detect habitat segregation among clones in the population would have been reduced.

Drivers of Zooplankton Patchiness

Sonar may also be used as a tool to the relative importance of abiotic versus biotic drivers of zooplankton patchiness (Folt and Burns 1999; Pinel-Alloul 1995; Zhou 1994). Abiotic factors that influence the spatial distribution of zooplankton include water movements (e.g., upwelling, Megard et al. 1997; Langmuir circulation, George and Edwards 1973), and thermal stratification (Pinel-Alloul 1988; Pinel-Alloul and Pont 1991). Diel vertical migration (Young and Watt 1996), predation avoidance (Pijankowska and Kowalczewski 1997), searching for food (Tiselius 1992), and locating mates (Strickler 1998) are among the biological forces that cause zooplankton to be patchily distributed (Folt and Burns1999). Patchiness is ecologically significant because it influences the interactions among individuals and populations. For example, extremely dense patches of grazing zooplankton can exert intense grazing pressure on a localized area (Hembre 2002a) and competition for food among individuals in a densely aggregated population will be stronger than in a population that is more evenly distributed. Also, interactions between predators (e.g., fish) and their zooplankton prey will be greatly affected by the ability of the fish to locate the patchily distributed zooplankton aggregations.

Our data from long-axis transects of Long Lake during the daytime in 1998 suggest that when the lake is stratified (May-October) dissolved oxygen concentrations (~ <1.5 mg/L) determine the lower limit of the distribution of the *Daphnia* population (Fig. 5). The upper limit of the distribution was below the mixed layer, but was less well defined.

Diel Pattern of Spatial Distribution

A comparison of the spatial distribution of *Daphnia* before, during, and after upward migration on 24 October, 1998 illustrates a diel component to patchiness (Fig. 9).

Before sunset, the population aggregated into a thin lens in the metalimnion between 15-16 m (Fig. 9 - panels A & B). Water below the *Daphnia* layer was anoxic, and depths above the aggregation were mixing (Fig. 9 - panel C). After sunset, the *Daphnia* population began to vertically migrate and to disperse into the mixed layer (Fig. 9 - panel A). After upward migration, the spatial distribution of the population was relatively homogeneous at depths above 16 meters (Fig. 9 - panels A & D), and volume backscattering was approximately two orders of magnitude less (-90 dB vs. -70 dB) than during the day.

This diel comparison of the spatial distribution of *Daphnia* sheds light on the relative importance of the abiotic and biotic factors that structure the population's spatial distribution. The depth range occupied by *Daphnia* during the day became progressively compressed from May to October as oxygen was depleted from deep water and the epilimnion thickened. At night, migration into the mixing waters of the epilimnion appeared to disperse the population. In October, dispersion into the epilimnion resulted in *Daphnia* becoming less concentrated because the volume of the epilimnion was much larger than the metalimnion. Early in the summer stratification period, however, when the volume of the epilimnion is less than that below it, *Daphnia* are likely to become more concentrated at night. These high concentrations would likely cause intense grazing on phytoplankton in the epilimnion and clear water.

The results of this study illustrate the practical utility of sonar for mapping the spatial distribution of zooplankton and for comprehensively assessing the size and biomass of populations. Our analysis also reveals the relative importance of abiotic and biotic drivers of patchiness in the spatial distribution of *D. pulicaria* during the ice-free

season of a dimictic lake. Abiotic factors delimited the range of depths in which *Daphnia* was distributed over the course of a day, while vertical migration behavior appeared to primarily determine the population's specific vertical location at any given time.

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Table 1. Data used to calibrate acoustic information. Z = net sampling interval depth (m); MVBS = mean volume backscattering (dB) in the sampling interval; Disp = volume of water displaced by sample; TS = large *Daphnia* target strength; LgDp = large-bodied *Daphnia* (>1.3 mm); SmDp = small-bodied *Daphnia* (≤ 1.3 mm); Cal = adult and copepodid calanoid copepods; Cyc = adult and copepodid cyclopoid copepods; Nau = copepod nauplii; Bos = *Bosmina*; Dia = *Diaphanosoma*. Body length data were not collected for samples from 1997 and 1996.

Sample		Volume		TS	Concentration (# m^{-3})							Mean Length (mm)					
Date (m/d/y)	Z (m)	MVBS (dB)	Disp (mL m^{-3})	LgDp (dB)	LgDp	SmDp	Cal	Cyc	Nau	Bos	Dia	Dp	Cal	Cyc	Nau	Bos	Dia
5/16/00	20-18	-73.5	18.3	-114	10100	2520	105	25500	733	0	0	1.80	0.82	0.68	0.18	-	-
5/16/00	18-16	-73.9	11.3	-116	14600	608	1920	6120	933	40	0	1.90	0.70	0.61	0.18	0.40	-
5/16/00	16-14	-72.9	20.9	-115	17600	4400	1360	28200	3250	0	0	1.85	0.85	0.58	0.21	-	-
5/16/00	14-12	-81.7	15.7	-124	16600	5260	3350	27600	1260	0	0	1.67	0.77	0.52	0.18	-	-
5/16/00	12-10	-81.2	6.1	-120	8000	1530	2620	12400	3350	0	0	1.76	0.92	0.48	0.21	-	-
5/16/00	10-8	-86.6	9.6	-128	13400	10600	18000	30100	8690	0	0	1.50	0.76	0.50	0.23	-	-
5/16/00	8-6	-113.3	5.2	-139	330	1240	37500	16400	8380	0	0	1.01	0.79	0.52	0.22	-	-
8/19/99	14-12	-79.3	20.1	-126	41900	7980	1050	25100	698	349	349	1.54	0.70	0.72	0.18	0.33	0.83
8/19/99	11-9	-95.2	3.5	-131	3360	13400	3320	12700	2270	1050	349	1.14	0.68	0.63	0.16	0.28	1.13
8/19/99	9-6	-110.2	0.6	-133	196	737	5930	6050	1860	2090	4420	1.14	1.01	0.44	0.17	0.33	0.99
7/10/99	18-17	-77.5	43.6	-125	51300	8360	5240	46100	698	0	0	1.86	0.79	0.64	0.20	-	-
7/10/99	16-13	-84.2	11.6	-126	16100	7584	7210	9190	233	0	0	1.68	0.86	0.61	0.25	-	-
5/31/99	22-19	-85.0	7.0	-120	3100	2070	1400	10700	175	0	0	1.54	0.68	0.63	0.20	-	-
5/31/99	19-16	-84.3	8.7	-119	3180	796	5080	8410	294	0	0	1.68	0.87	0.62	0.19	-	-
5/31/99	15-12	-94.2	7.6	-127	2000	4270	22800	8800	2610	0	0	1.31	0.76	0.58	0.20	-	-
5/31/99	12-9	-93.3	5.8	-123	866	3460	10600	7510	3100	0	0	1.10	0.88	0.46	0.18	-	-
5/31/99	8-4	-94.5	1.8	-122	544	2180	15100	3010	2030	0	0	1.02	0.77	0.49	0.15	-	-
5/31/99	22-0	-90.3	4.8	-126	3300	2600	24200	9850	1600	0	67	1.51	0.73	0.51	0.20	-	1.10
4/24/99	18-16	-77.3	14.0	-118	10900	946	419	5970	1570	0	0	2.11	0.73	0.67	0.19	-	-
4/24/99	12-9	-108.7	4.7	-132	189	567	6860	17630	4480	0	0	1.13	0.86	0.45	0.19	-	-
10/24/98	16-13	-86.5	8.1	-121	2770	3120	776	68600	155	155	0	1.75	1.08	0.88	0.19	0.30	-
10/24/98	22-0	-108.1	1.7	-135	482	206	4150	16600	370	291	0	1.70	1.20	0.90	0.20	0.40	-
8/22/98	16-14	-83.9	-	-124	10100	3650	1470	21800	2300	0	209	1.70	0.85	0.60	0.19	-	0.85
8/22/98	13-11	-82.5	-	-116	2340	1260	13700	11100	1090	109	1640	1.61	0.93	0.53	0.19	0.35	0.96
8/22/98	4-2	-97.2	0.9	-117	101	248	1510	756	2850	0	291	1.41	0.57	0.63	0.2	-	0.52
7/25/98	19-16	-76.8	11.1	-118	14400	1600	1020	26600	150	0	0	1.94	0.88	0.78	0.20	-	-
7/25/98	15-13	-80.9	9.6	-117	3880	4750	5240	21600	1310	130	0	1.49	0.83	0.59	0.18	0.30	-
7/25/98	13-10	-84.6	2.9	-113	679	6110	30200	20800	1070	388	97	1.12	0.82	0.54	0.18	0.30	0.85
7/25/98	22-0	-86.3	-	-121	3050	2040	5500	1170	381	167	24	1.64	0.77	0.71	0.20	0.33	1.00
5/25/98	22-18	-71.9	9.6	-108	3600	538	576	5860	1360	0	0	2.30	0.86	0.74	0.20	-	-

Table 1. cont.

Sample		Volume		TS	Concentration (# m^{-3})							Mean Length (mm)					
Date (m/d/y)	Z (m)	MVBS (dB)	Disp (mL m^{-3})	LgDp (dB)	LgDp	SmDp	Cal	Cyc	Nau	Bos	Dia	Dp	Cal	Cyc	Nau	Bos	Dia
5/25/98	18-14	-72.2	-	-110	5600	2060	1880	6810	1730	0	0	2.07	1.04	0.61	0.20	-	-
5/25/98	13-11	-69.2	20.9	-110	11200	6020	4400	9840	2620	0	0	1.79	1.02	0.58	0.20	-	-
5/25/98	11-9	-71.3	-	-115	23400	7390	5650	7120	4400	0	0	1.67	0.73	0.52	0.20	-	-
5/25/98	9-5	-76.7	-	-108	1490	19800	13900	9010	4030	0	0	1.59	0.84	0.52	0.20	-	-
5/25/98	22-0	-73.1	4.1	-110	4970	740	3970	2790	190	0	0	1.78	0.85	0.61	0.20	-	-
4/22/98	23-19	-97.4	0.9	-121	244	570	291	9250	465	0	0	1.30	1.05	0.83	0.20	-	-
4/22/98	15-11	-99.8	1.3	-123	227	1280	2160	29600	3340	0	0	1.37	1.05	0.54	0.20	-	-
4/22/98	11-7	-95.9	1.3	-121	298	942	1240	2160	945	0	0	1.30	1.02	0.53	0.20	-	-
4/22/98	7-3	-81.7	-	-112	1180	3740	2170	75800	8130	0	0	1.30	1.08	0.56	0.21	-	-
8/7/97	13-10	-81.8	-	-118	4210	2070	11000	19600	1940	0	465	-	-	-	-	-	-
9/21/97n	12.5-11	-86.4	-	-125	7300	4100	3580	24900	2490	0	0	-	-	-	-	-	-
9/21/97n	10-8	-85.7	-	-119	2180	91	3400	7240	1050	349	0	-	-	-	-	-	-
9/21/97n	7-2	-73.0	-	-112	8400	350	10530	2270	378	972	216	-	-	-	-	-	-
8/7/97n	11-8	-76.0	-	-115	7770	3830	5850	23500	1400	0	87	-	-	-	-	-	-
8/7/97n	8-5	-79.2	-	-117	5790	1450	23700	25600	838	0	8120	-	-	-	-	-	-
10/11/96	20-15	-96.6	-	-122	349	349	4610	14700	1120	1530	0	-	-	-	-	-	-
10/11/96	13-11	-80.6	-	-116	3380	0	3210	23300	22230	1820	0	-	-	-	-	-	-
10/11/96	10-7	-92.7	-	-117	256	256	18900	4980	1400	6890	0	-	-	-	-	-	-
10/11/96	22-0	-91.4	-	-120	686	76	9110	7760	571	2670	0	-	-	-	-	-	-
MEAN		**-85.6**	**9.2**	**-120**	**7120**	**3260**	**7510**	**16700**	**2420**	**388**	**333**	**1.57**	**0.86**	**0.60**	**0.19**	**0.36**	**0.81**

Table 2a. Multiple linear regression of volume backscattering (dB) versus 10 Log_{10} concentration (# m^{-3}) of all zooplankton taxa. The regression is significant ($p < 0.0001$) and the R^2 is 0.67.

Predictor variable	Coefficient	Std. Error	Student's T	P-value
Constant	-104.1	13.19	-7.89	< 0.0000
Large *Daphnia*	1.343	0.164	8.17	< 0.0000
Small *Daphnia*	-0.110	0.176	-0.63	0.533
Calanoid	-0.217	0.202	-1.08	0.389
Cyclopoid	-0.445	0.262	-1.70	0.097
Nauplii	0.061	0.225	0.27	0.787
Diaphanosoma	-0.018	0.090	-0.20	0.842
Bosmina	-0.027	0.091	-0.30	0.765

Source	DF	SS	MS	F	P-value
Regression	7	3842	548.8	11.97	< 0.0001
Residual	41	1880	45.85		
Total	48	5722			

Table 2b. Simple linear regression of volume backscattering (dB) versus 10 Log_{10} concentration (# m^{-3}) of large *Daphnia*. The regression is significant ($p < 0.0001$) and the R^2 is 0.63.

Predictor variable	Coefficient	Std. Error	Student's T	P-value
Constant	-128.8	4.966	-25.93	< 0.0001
Large *Daphnia*	1.254	0.142	8.86	< 0.0001

Source	DF	SS	MS	F	P-value
Regression	1	3578	3578	78.44	< 0.0001
Residual	47	2144	45.61		
Total	48	5722			

Table 3. Multiple linear regression of volume backscattering (dB) versus Log_{10} (x+1) biomass ($\mu g\ m^{-3}$) of all zooplankton taxa. The regression is significant ($p < 0.0001$) and the R^2 is 0.79.

Predictor variable	Coefficient	Std. Error	Student's T	P-value
Constant	-124.9	13.16	-9.49	< 0.0000
Daphnia	14.11	1.494	9.44	< 0.0000
Calanoid	-2.692	1.838	-1.46	0.153
Cyclopoid	-4.463	2.254	-1.98	0.056
Nauplii	-1.166	2.244	-0.52	0.607
Diaphanosoma	-0.548	1.091	-0.50	0.619
Bosmina	-0.978	1.703	-0.57	0.570

Source	DF	SS	MS	F	P-value
Regression	6	4084	680.7	19.89	< 0.0001
Residual	32	1095	34.2		
Total	38	5179			

Table 4. *Daphnia* population size and concentration inferred from acoustic information. Concentration was calculated by dividing the total number of *Daphnia* equivalents by the lake volume.

Date (1998)	Population Size (equivalents)		Concentration (equivalents m^{-3})	
	N	s.e.	N	s.e.
22 April	4.85×10^{10}	1.30×10^{10}	6360	1700
25 May	4.81×10^{11}	8.81×10^{10}	63100	11600
27 June	2.95×10^{10}	1.08×10^{10}	3860	1420
10 July	2.63×10^{9}	9.40×10^{8}	345	123
22 August	1.29×10^{10}	4.04×10^{9}	1690	530
24 October	1.77×10^{9}	6.28×10^{8}	232	82

Figure Legends

Figure 1. Layers of sound scatterers detected with sonar while anchored at the sampling station. Acoustic data collected in this manner were compared to zooplankton densities from net samples to calibrate the sonar information. The white rectangles superimposed on this echogram indicate the depths that were sampled with vertical tows with plankton net (19 August, 1999).

Figure 2. Echogram from a transect along the long axis of Long Lake during daylight, 25 May, 1998.

Figure 3. Variation of mean volume backscattering strength along the long axis of the lake at three depths during daylight, 25 May, 1998.

Figure 4. Spatial variation of backscattered sound along the long axis during spring, summer and autumn, 1998.

Figure 5. Temperature and oxygen isopleth diagrams showing the spatial extent of the deep scattering layer (shaded) in 1998.

Figure 6. Dependence of mean volume backscattering strength (left) and plankton displacement (right) on the concentration of large *Daphnia*, small *Daphnia* and other planktonic crustaceans. Volume backscattering strength and displacement volume depend most strongly on the concentration of large *Daphnia*.

Figure 7. Linear regression of mean volume backscattering strength (dB) on total biomass of *Daphnia* (μg dry mass m^{-3}).

Figure 8. Proportions of taxa that composed the zooplankton biomass in 1998. *Daphnia* dominated the zooplankton community until the fall when copepods became abundant. Nauplii and 'other Cladocera' comprised very little of the zooplankton biomass throughout the year.

Figure 9. Composite figure showing the diel spatial distribution of zooplankton on 24 October, 1998. The data presented in the top panel (A) were recorded from a stationary position in 22 m of water. This panel shows the spatial distribution of the *Daphnia* layer (15-16 m) just before sunset, and the upward migration and dispersion of the layer after sunset until 8 p.m. CST. On this date, the *Daphnia* population was densely aggregated in a thin layer in the metalimnion across the lake during the day (long-axis transect echogram - panel B). The layer was bounded by anoxic water at depths below 16 m, and the mixed layer above 15 m (panel C). Acoustic data recorded while traveling from the sampling location to the west end of the lake after migration (panel D) illustrates that the dispersion of the daytime *Daphnia* layer was not a localized phenomenon.

Figure 1.

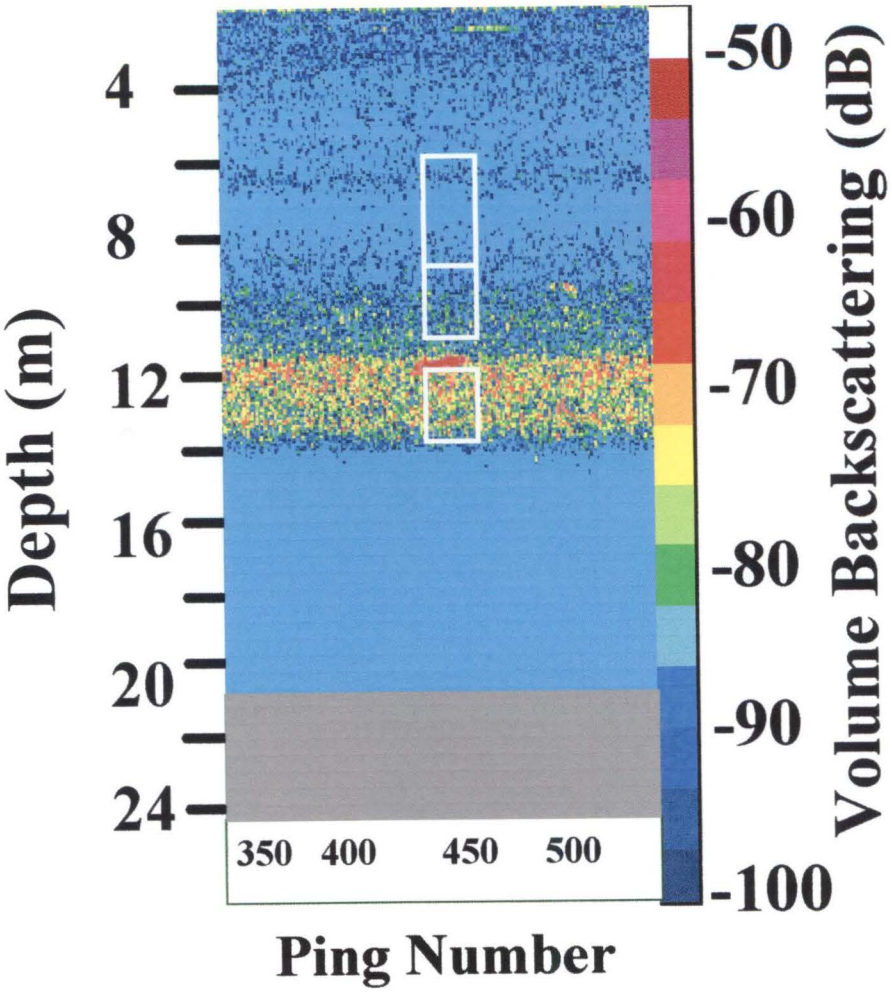

Depth (m)
4
8
12
16
20
24
350
400
450
500
Ping Number
-50
-60
-70
-80
-90
-100
Volume Backscattering (dB)

Figure 2.

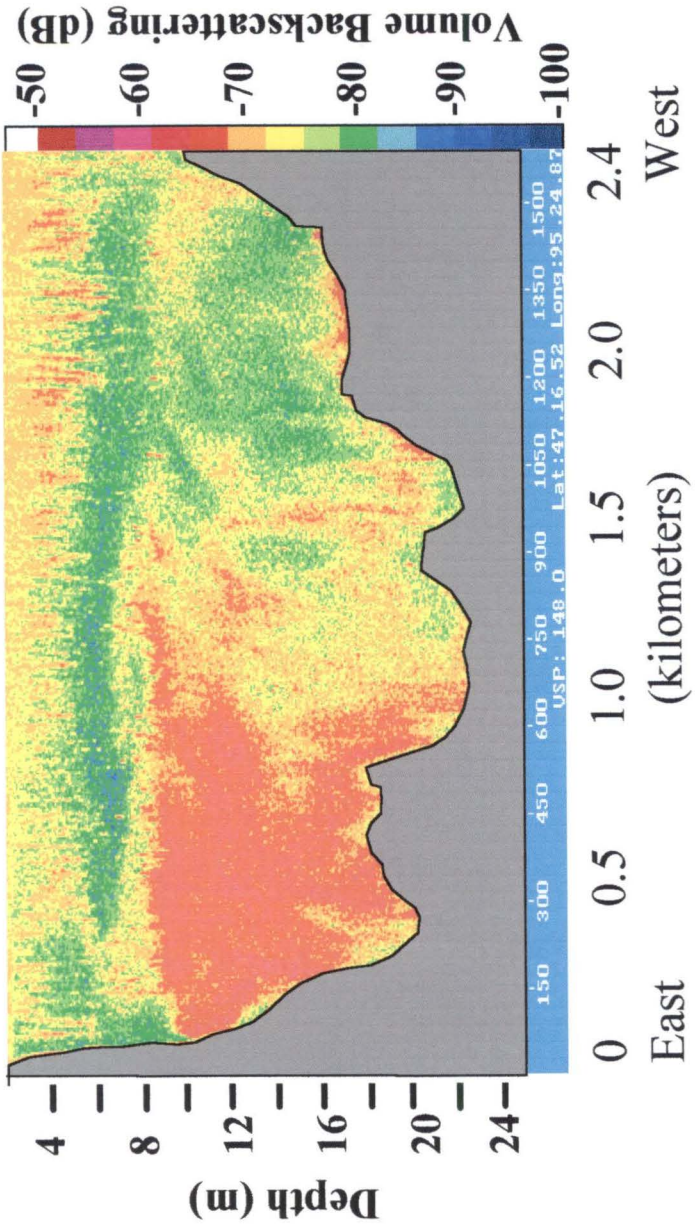

Volume Backscattering (dB)
-50
-60
-70
-80
-90
-100
Depth (m)
4
8
12
16
20
24
0
0.5
1.0
1.5
2.0
2.4
East
(kilometers)
West
150 300 450 600 750 900 1050 1200 1350 1500
USP: 148.0 Lat:47.16.52 Long:95.24.87

Figure 3.

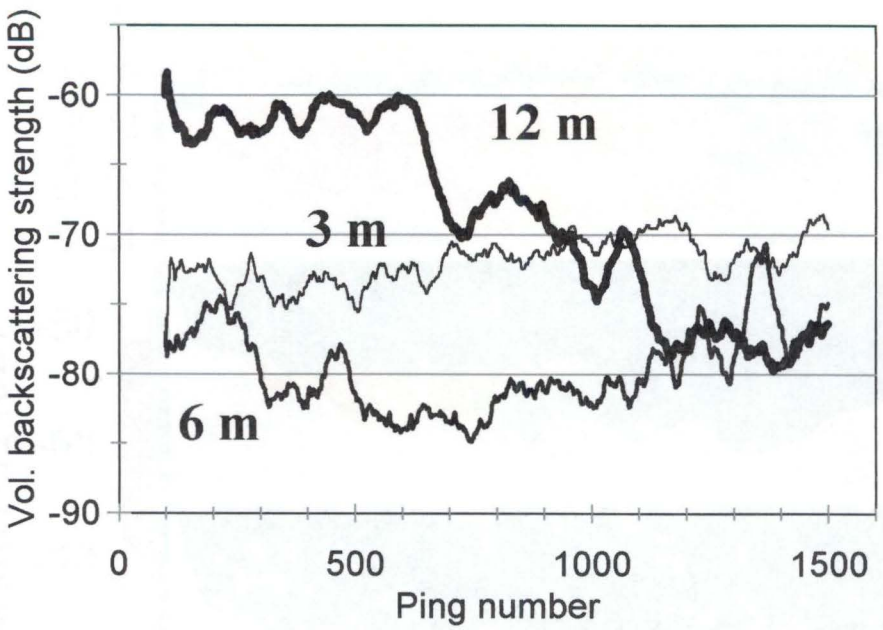
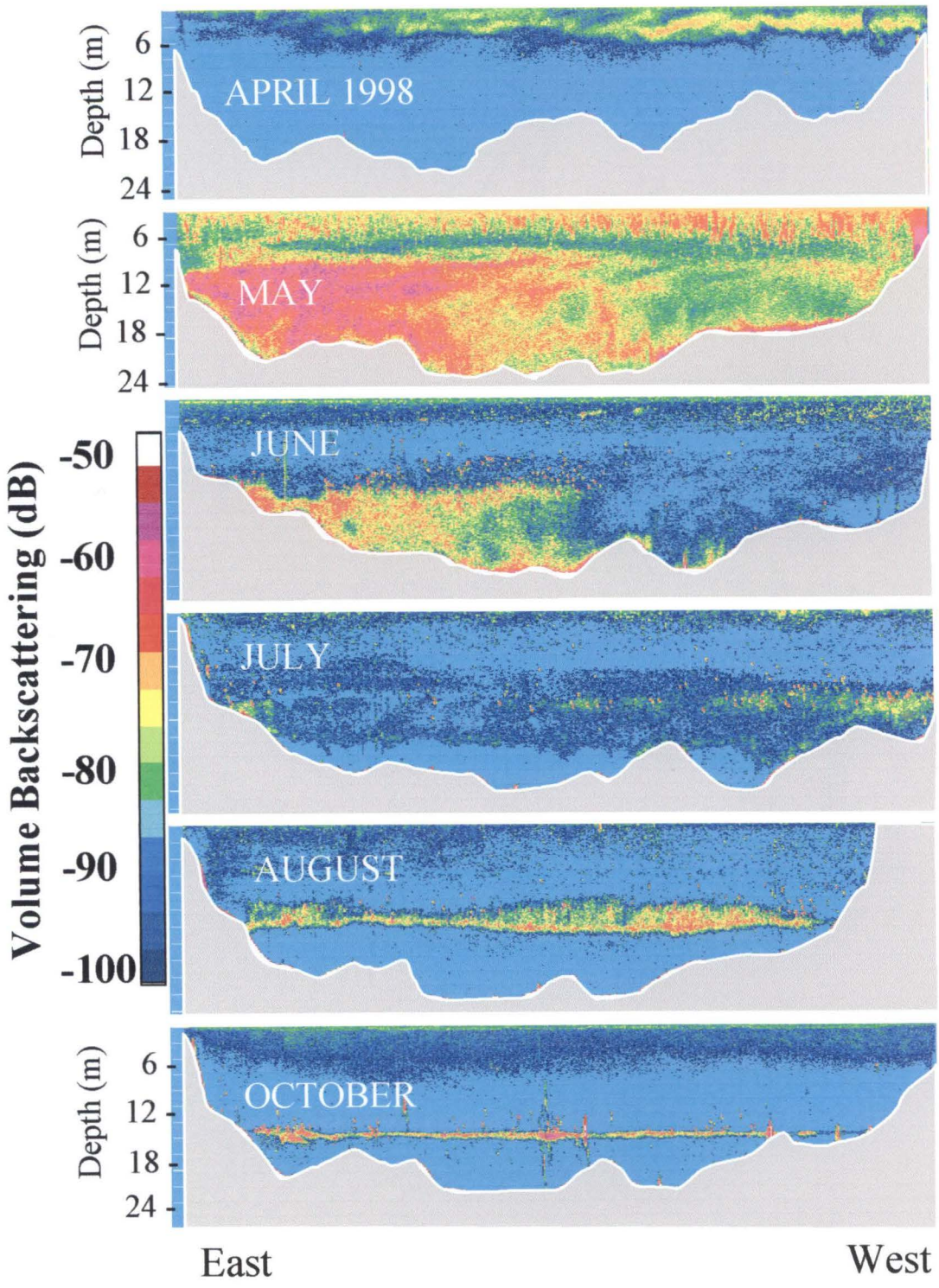

Vol. backscattering strength (dB)
-60
-70
-80
-90
12 m
3 m
6 m
0
500
1000
1500
Ping number

Figure 4.


Depth (m)
6
12
18
24
APRIL 1998
MAY
JUNE
JULY
AUGUST
OCTOBER
Volume Backscattering (dB)
-50
-60
-70
-80
-90
-100
East
West

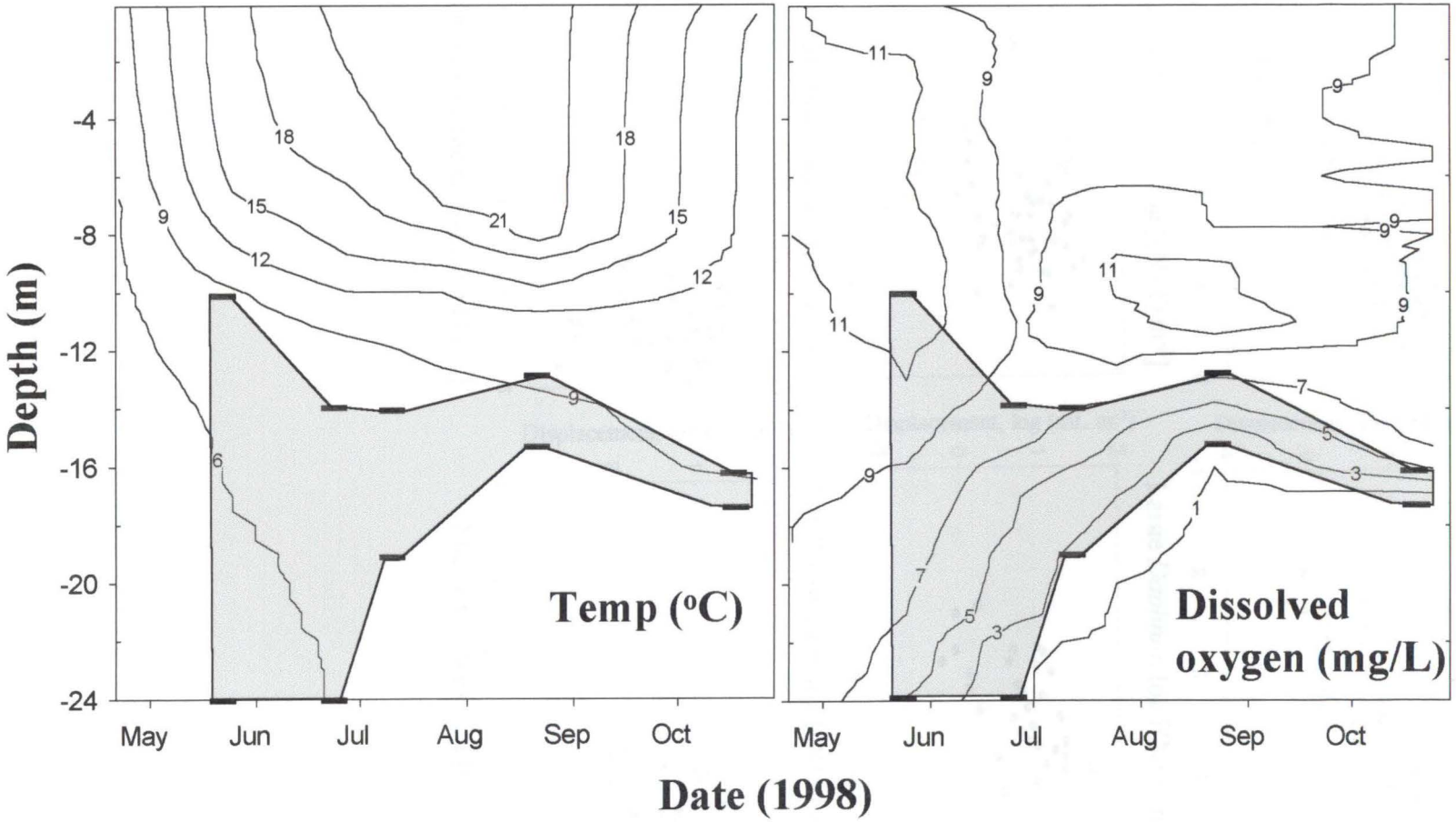

Depth (m)
-4
-8
-12
-16
-20
-24
May
Jun
Jul
Aug
Sep
Oct
Temp (°C)
Dissolved oxygen (mg/L)
Date (1998)

Figure 5.

Figure 6.

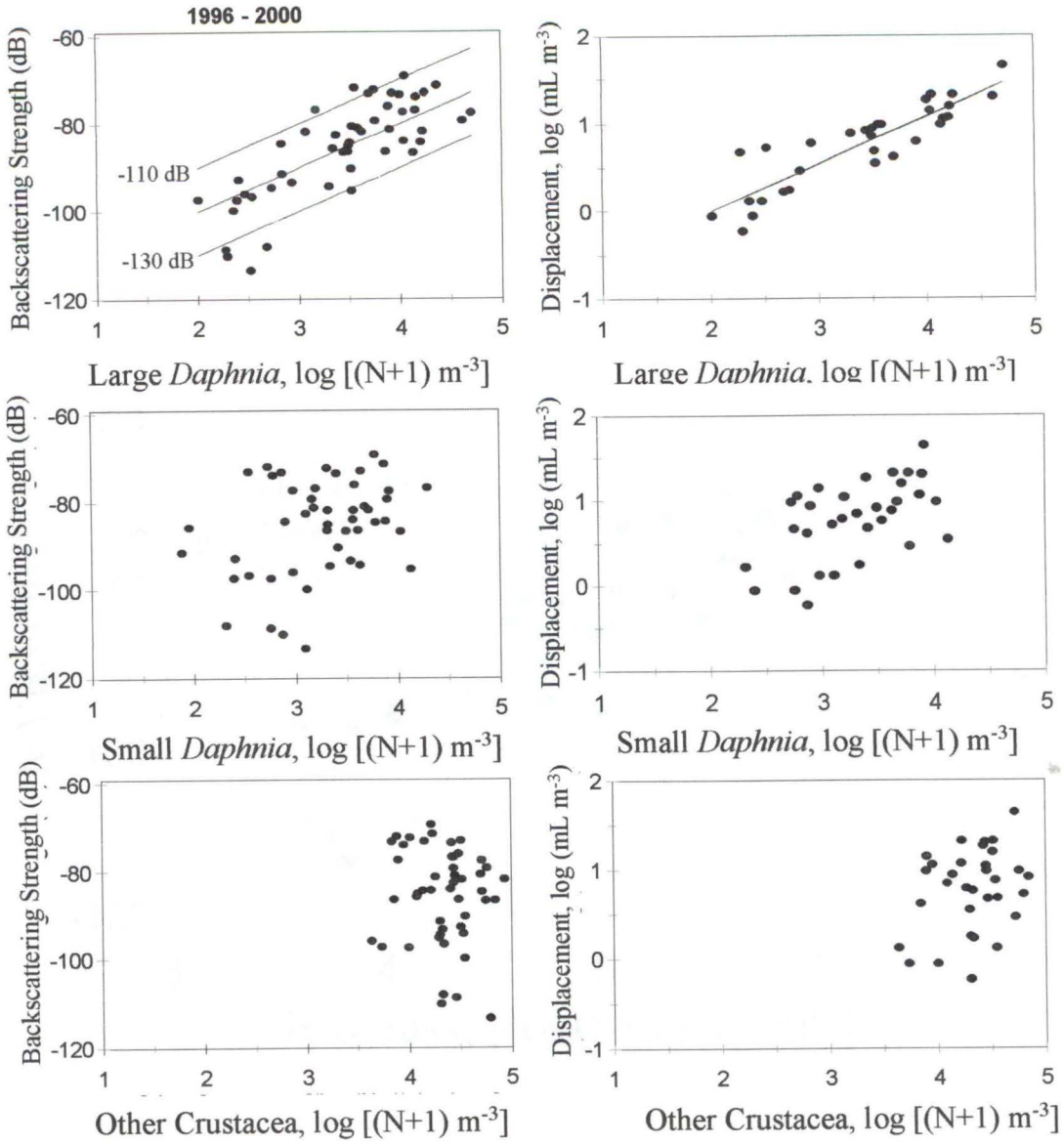

1996 - 2000
Backscattering Strength (dB)
-60
-80
-100
-120
-110 dB
-130 dB
1
2
3
4
5
Large *Daphnia*, log [(N+1) m^{-3}]
Displacement, log (mL m^{-3})
2
1
0
-1
Large *Daphnia*, log [(N+1) m^{-3}]
Small *Daphnia*, log [(N+1) m^{-3}]
Small *Daphnia*, log [(N+1) m^{-3}]
Other Crustacea, log [(N+1) m^{-3}]
Other Crustacea, log [(N+1) m^{-3}]

Figure 7.

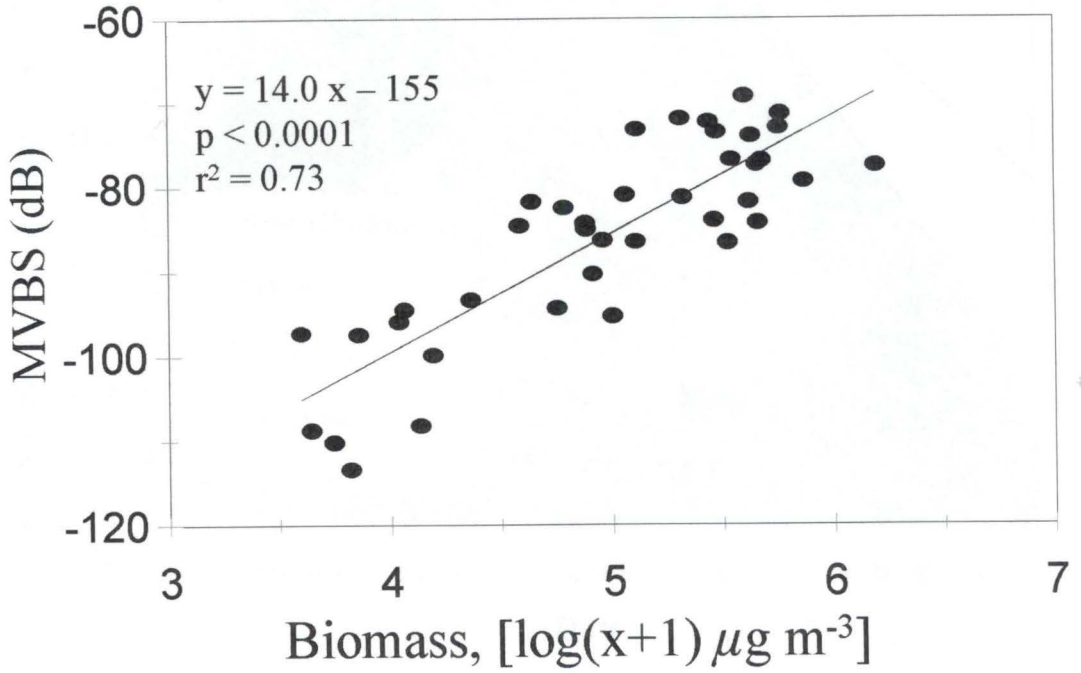
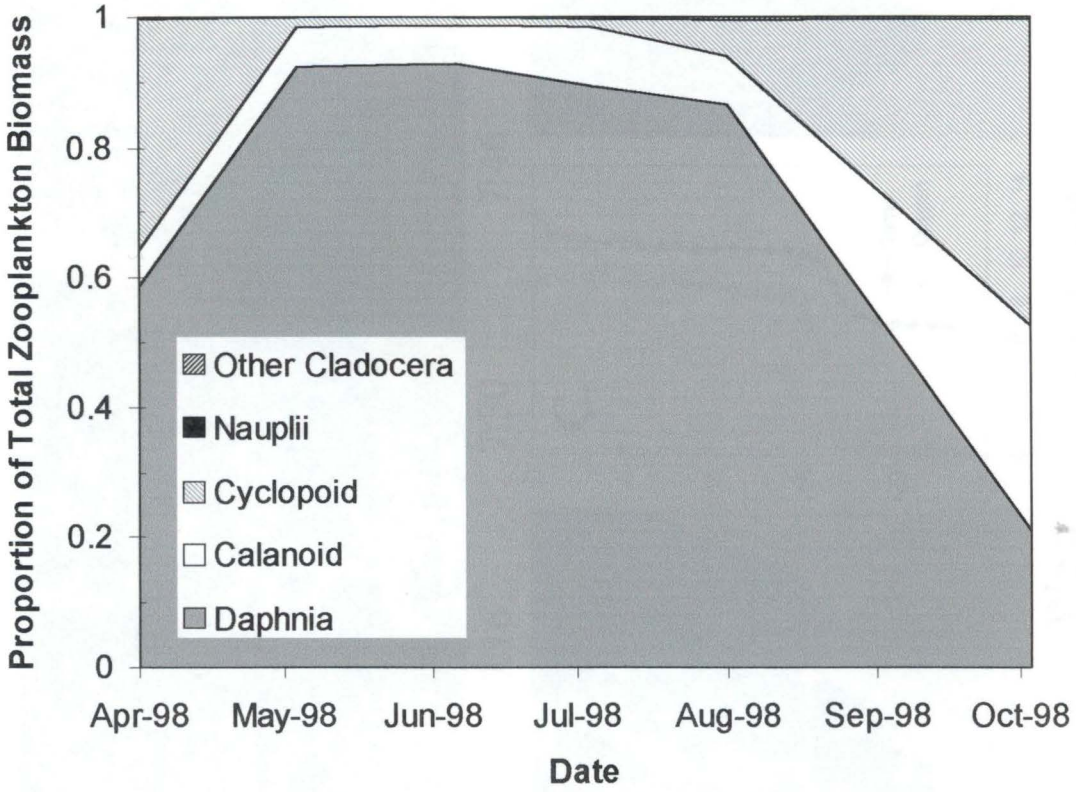

-60
-80
-100
-120
MVBS (dB)
y = 14.0 x – 155
p < 0.0001
r² = 0.73
3
4
5
6
7
Biomass, [log(x+1) μg m⁻³]

Figure 8.


Proportion of Total Zooplankton Biomass
1
0.8
0.6
0.4
0.2
0
Other Cladocera
Nauplii
Cyclopoid
Calanoid
Daphnia
Apr-98
May-98
Jun-98
Jul-98
Aug-98
Sep-98
Oct-98
Date

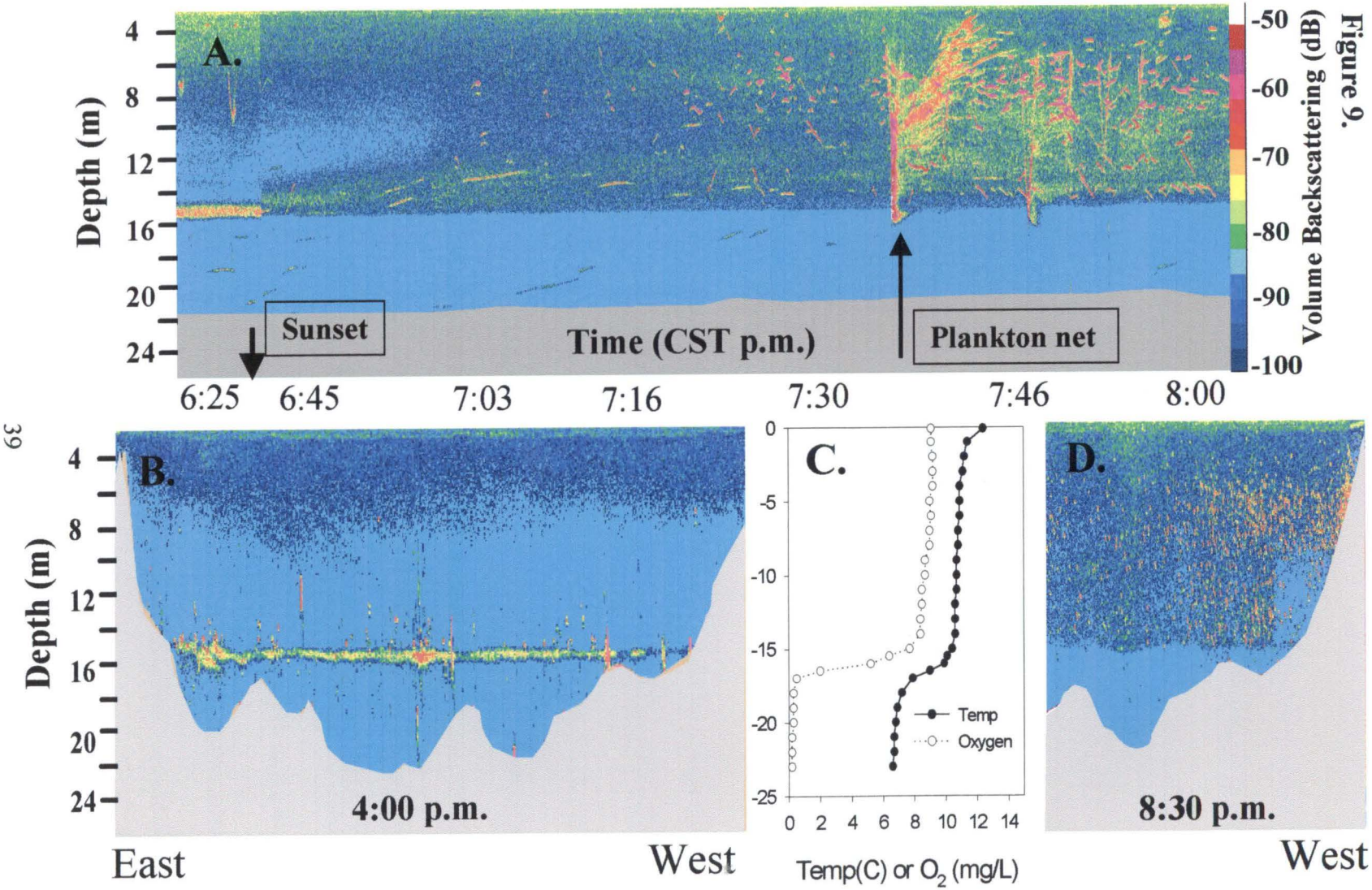

A.
Depth (m)
4
8
12
16
20
24
Sunset
Time (CST p.m.)
Plankton net
6:25
6:45
7:03
7:16
7:30
7:46
8:00
-50
-60
-70
-80
-90
-100
Volume Backscattering (dB)
B.
4:00 p.m.
East
West
C.
0
-5
-10
-15
-20
-25
0 2 4 6 8 10 12 14
Temp
Oxygen
Temp(C) or O_2 (mg/L)
D.
8:30 p.m.
West

Figure 9.

CHAPTER 2: Dependence of *Daphnia* Demography and Water Clarity on the Timing of Trout Predation

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Abstract

The effect of rainbow trout on a population of *Daphnia pulicaria* and water clarity of a Minnesota lake was studied for four years (1996-1999). Trout were stocked in the autumn for the first two years and in the spring for the second two years. The sizes of both *Daphnia* & trout populations were estimated with sonar. In years when trout were stocked in the fall (1996-1997), predation by trout over the winter nearly eliminated *Daphnia* by spring. In both fall-stocking years, the *Daphnia* population increased as the trout population diminished during the summer. Trout were not stocked in the fall of 1997, but were instead added to the lake in the spring of 1998 soon after the ice melted, and again in the spring of 1999. The change resulted in more predation on *D. pulicaria* during the spring, summer, and autumn. However, high predation was offset by high rates of reproduction in the spring by the large, fecund *Daphnia* population that survived the winter. The *Daphnia* population grew by an order of magnitude, and its grazing produced spring clear-water phases that were inhibited when trout were stocked in the autumn.

The outcomes of the fisheries manipulation achieved seemingly mutually exclusive objectives: a robust sport fishery of a planktivorous fish, and clear water for other forms of recreation. The large late spring-early summer *Daphnia* population provided both clear water (as a result of their feeding) and abundant food for the trout.

Introduction

Large-bodied *Daphnia* play an important role in the pelagic food web of lakes. They are effective grazers of phytoplankton, and when abundant can dramatically reduce the standing crop of algae (Shapiro and Wright 1984; Lampert et al. 1986; Carpenter et al. 1987). They are also often the preferred prey of zooplanktivorous fish, which are visual predators and selectively feed on large zooplankton (e.g., Hrbacek et al. 1961; Brooks and Dodson 1965; Post and McQueen 1987; Luecke et al. 1990; Rudstam et al. 1993). Lake managers have exploited these trophic interactions as a means to reduce phytoplankton biomass in a process known as "biomanipulation" (Shapiro and Wright 1984). In successful biomanipulation efforts, the abundance of zooplanktivorous fish is reduced (either through direct removal, or through the stocking of piscivorous fish), herbivorous zooplankton biomass increases, phytoplankton biomass decreases, and water clarity increases (Brett and Goldman 1996; Meijer et al. 1999).

Often, fisheries management efforts are consistent with water quality concerns. For example, the stocking of piscivorous game fish (e.g., walleye, pike, bass, etc.) may initiate a trophic cascade (Carpenter et al. 1987) that reduces the abundance of zooplanktivorous fish, increases the abundance of large-bodied zooplankton (e.g., *Daphnia*), decreases phytoplankton biomass, and increases water clarity. However, when the game fish to be stocked is a zooplanktivore there is a potential conflict between the providing a sport fishery, and maintaining clear water. According to tenets of trophic cascade theory, the addition of planktivorous fish is expected to decrease the abundance of large-bodied zooplankton (e.g., large *Daphnia*), and result in higher levels of algae and lower water transparency.

Rainbow trout (*Oncorhynchus mykiss*) is an example of a zooplanktivorous fish that is commonly stocked in lakes to provide a cold-water sport fishery. Several studies have examined the impact of rainbow trout (RBT) predation on *Daphnia*. Most of these studies (Galbraith 1967; Taylor and Gerking 1980; Wang et al. 1996; Hirsch and Negus 2000) focused on the trout fishery and aimed to determine the diet of the trout and whether the lakes could support a population of stocked trout. Two studies, however, have explicitly examined how perturbations in RBT population density affects phytoplankton biomass and water clarity (Geist et al. 1993; Elser et al. 1995).

Although RBT was a size-selective predator on *Daphnia* in all studies, the strength of the predator-prey relationship between RBT and *Daphnia* differed. Galbraith (1967) and Hirsch and Negus (2000) found that RBT preferentially fed on *Daphnia* and were size-selective (i.e., usually only consumed *Daphnia* > 1.3 mm in length). In one of the lakes studied by Galbraith (1967), the introduction of RBT led to the elimination of *D. pulex* within four years. Hirsch and Negus (2000) concluded that while RBT selectively preyed on *Daphnia*, the level at which the fish were stocked was not high enough to substantially lower *Daphnia* densities.

Taylor and Gerking (1980), and Wang et al. (1996) also found that *Daphnia* consumed by RBT were consistently larger than 1.3 mm, but the fish did not show the same preference for *Daphnia* as in the previously mentioned studies. In both of these studies, RBT typically had a mixed diet of benthic (e.g., amphipods, dipterans) & planktonic organisms. In the study by Wang et al. (1996), chironomid midge larvae and pupae replaced *Daphnia* as the primary diet component of the trout by mid-summer, with *Daphnia* only comprising 3% of the trout diet by August. The authors attribute this shift

in diet to the inability of trout to forage for *Daphnia* in deep water with oxygen concentrations below 5 mg/L.

The two studies that explicitly investigated the effect of RBT predation on the biomass of primary producers had contradictory findings. Geist et al. (1993) analyzed an 8-year data set from a lake in Washington and found results that were consistent with expectations of top-down control on the phytoplankton standing crop. Multiple regession analyses revealed that fluctuations in RBT abundance had a greater effect on phytoplankton standing crop than did total phosphorus levels.

Elser et al. (1995) examined how discontinuing the stocking of rainbow trout to an oligotrophic lake (Castle Lake, northern California) affected food web interactions and ecosystem properties (e.g., light penetration, primary productivity) of the lake. Contrary to the findings of Geist et al. (1993) and expectations of trophic cascade theory, Elser et al. found that the reduction of rainbow trout abundance resulted in unexpected compensatory increases in the abundance of other zooplanktivorous fish species. This indirect effect caused increased planktivory on daphnids, increased primary productivity, and decreased water clarity.

These conflicting results illustrate that there is not a consensus on the effect of RBT on *Daphnia* populations. There is also uncertainty about how the predator-prey interaction affects phytoplankton abundance and water clarity. Uncertainty about these issues is a serious impediment to our ability to manage fisheries and water quality.

In this study we examine the consequences of two different ways of stocking trout. In the first two years (1996-1997) trout were stocked in the fall, and in the second two years (1998-1999) they were stocked in the spring, soon after ice-out. Specifically

we aimed to answer the following questions: 1) Does predation by rainbow trout significantly affect the demography of the *Daphnia* population in the lake? 2) Did the change from autumn to spring stocking alter the nature of the trophic interactions among trout, *Daphnia*, and phytoplankton in the lake? and 3) What is the appropriate stocking strategy to maximize trout survival and also maintain clear water?

To examine these questions we monitored the abundance and spatial distribution of *Daphnia* and trout with high-frequency sonar and conventional sampling. Phytoplankton abundance was measured with chlorophyll a, and transparency was measured with a secchi disk.

Study Site

Long Lake is a single-basin, dimictic lake located in northwestern Minnesota (latitude 47 17' N, longitude 95 17' W). The lake is 2.4 km long, has a surface area of 66.5 hectares, a maximum depth of 24 m, an average depth of 13 m, and a volume of 7.63 x 10^6 m^3 (Fig. 1). The lake basin has steep slopes, a small littoral zone, and very little emergent or submergent macrophyte growth. It has no inflowing streams and receives its water from springs and direct drainage. There is a single outlet stream at the southeast end of the lake. Historically, the lake has been more transparent than other lakes in the area, with secchi transparencies of up to 10.5 m (Moyle 1969). During summer stratification, maximum oxygen concentrations typically occur in the metalimnion. The cold, well-oxygenated waters of the metalimnion provide ideal habitat for the rainbow trout (*Oncorhynchus mykiss)* that have been stocked annually by the Minnesota Department of Natural Resources (MDNR) since 1961.

Fisheries Management

Long Lake is one of more than 150 lakes in Minnesota that are stocked with stream trout by the MDNR. Although the lake contains several native planktivores including, yellow perch (*Perca flavescens*), bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) sunfish, and minnow species (Cyprinidae) (MDNR fish survey 1988), the addition of rainbow trout, a pelagic planktivore, is a perturbation that increases predation on *Daphnia* above its natural limits. Rainbow trout require streams with current-washed gravel to spawn, and since such streams are not available to the trout in Long Lake, the abundance of trout is not affected by reproduction. Instead, trout abundance is determined by the number of fish that are stocked, natural mortality, and mortality due to fishing.

For the first two years of this study (1996-1997), trout were stocked the preceding fall as fingerlings. The quality of trout fishing was poor in the summers of 1996 and 1997 (MDNR personal comm.). In an effort to improve fishing, the MDNR decided to change its management strategy. The same number of fish (14,500) was stocked in 1998 and 1999 as in 1996 and 1997 (Table 1), but the fish were held over the winter in the hatchery and stocked in the spring soon after ice-out (April 23, 1998 and May 2, 1999).

Methods

Use of Sonar System

To obtain information about the distribution and abundance of zooplankton and fish, we employed a sonar system (Megard et al. 1997) that consists of a Lowrance X-16 high-frequency (192 kHz) single-beam echosounder and a Loran-C navigation receiver connected to an IBM personal computer. An analog converter digitizes voltage variation due to backscattered sound from zooplankton aggregations and other sound-scatterers. The software for the system (Megard et al. 1997) uses the sonar equations (e.g., Urick 1983) to transform the digitized echo strengths to volume scattering strengths. The volume scattering strengths are displayed instantaneously on the computer monitor as echograms, and the echograms were used to locate aggregations of zooplankton and to select depth increments for net sampling. In addition to providing instantaneous information for plankton net sampling, the sonar data were saved on the computer's hard disk and later analyzed to estimate the abundance of *Daphnia* in the lake. For details about the configuration of the system and its operation see chapter 1.

Estimation of Daphnia Abundance

To evaluate zooplankton distribution and abundance, a narrow beam (4° half angle) transducer was directed vertically from the bow of the boat, and acoustic data were collected while traveling at about 5 km h^{-1} along a transect of the lake's long axis from the southeast to the northwest end of the lake (Fig. 2). Sonar data from long-axis transects were collected on five dates in 1996 (12 June, 18 July, 1 August, 26 August, and 11 October), four dates in 1997 (16 June, 7 July, 7 August, 21 September), six dates in 1998 (22 April, 25 May, 27 June, 25 July, 22 August, and 24 October), and five dates in 1999

(24 April, 31 May, 10 July, 19 August, and 2 October). On two sampling dates (31 May and 27 June 1996) no sonar data were collected, and on two other dates (17 May 1997 and 27 June 1999) the data for the long-axis transect were lost due to electronic malfunctions. For these four dates, *Daphnia* density was measured only by conventional sampling with a plankton net. On all other dates, we computed *Daphnia* population density from sonar information collected from the long-axis transects, using a *Daphnia* target strength of -120 dB (Chapter 1). *Daphnia* population size was calculated from the sonar information in the following way. The mean volume backscattering (dB) at depths where *Daphnia* occurred was divided by the target strength to provide an acoustic estimate of the concentration of "*Daphnia* equivalents" at different depths. The water volumes of the relevant depth intervals were then multiplied by the concentrations of *Daphnia* equivalents, and the sum of these products was taken as a measure of the total number of *Daphnia* in the lake (Fig. 3).

Estimation of Trout Abundance

On 13 dates we performed a series of "zigzag" transects (Fig. 2) using a wide-beam (10^{o} half angle) transducer to obtain information about the abundance of rainbow trout. This transducer was used for fish surveys because the wider beam angle provides a clearer image of arc-shaped fish echoes than does the narrow-beam transducer. To estimate trout abundance, fish echoes were counted from echograms at depths within the bounds of the rainbow trout habitat (i.e., depths where the temperature was $\leq 21^{o}$ C, and oxygen concentrations were ≥ 5 mg/L, (Wang et al. 1996). On dates when zigzag transects were not done, we estimated trout abundance by counting fish echoes from the long-axis transects obtained using the narrow beam transducer (Fig. 4). For these

transects, we estimated trout abundance by filtering out the weaker echoes (< -70 dB) and counting fish traces in the depth interval containing viable trout habitat.

To standardize the two sonar methods for assessing trout abundance, counts from the zigzag transects were regressed against those from the long-axis transects. The relationship was highly significant (p-value < 0.0001) with an R^2 of 0.91 (Fig. 5 - upper panel). We used the standardized data from 1998 to relate echogram counts of trout echoes to the actual number of rainbow trout in the lake. These data were used because they include a measure of trout abundance on the day before trout were stocked (22 April 1998), and a series of dates after 14,500 trout were added. We assumed that the count of trout echoes obtained from the 22 April, 1998 long-axis transect represented zero, or "trace" levels of trout because trout had not been stocked to the lake for 17 months (since November of 1996). The y-intercept value of the linear regression of standardized counts from the May-October sampling dates in 1998 versus the number of days after trout were stocked (23 April) was taken as an estimate of the standardized count value equivalent to 14,500 trout (Fig. 5 - middle panel). Finally, to translate the standardized sonar estimates of trout counts to an estimate of the actual number of trout in the lake, we extrapolated between the standardized count value presumed to represent zero or trace levels of trout, and the value presumed to represent 14,500 trout (Fig. 5 - lower panel).

Environmental Sampling

We sampled Long Lake approximately monthly during the open-water seasons of 1996-1999. Sampling was conducted during the day (between 11 a.m. and 5 p.m.) at a deep (22-24 m) location in the middle of the lake (Fig. 2). Secchi transparency, and

vertical profiles of temperature, oxygen, and turbidity were obtained at the sampling site. Supplemental water transparency data was obtained from the University of Minnesota's Lake Itasca Biological Station for 1996, and from the Minnesota Department of Natural Resources for 1997-1999. Temperature and dissolved oxygen were measured at 1-m intervals with a YSI model 58 dissolved oxygen meter. In 1996 and 1997, turbidity was measured at 1-m intervals with a Montedoro-Whitney transmissometer.

Zooplankton Sampling

Depth intervals were selected with the aid of sonar and sampled with vertical tows of a closing Wisconsin-style plankton net (27 cm diameter, 130 μm mesh size). The bucket of the net was fitted with a 130 μm filter to collect zooplankton for enumeration and sonar calibration (Chapter 1). Zooplankton samples were preserved in the field with a chilled sucrose-formalin solution (Prepas 1978) and refrigerated until they were analyzed. For each sample, animals in five 5 mL subsamples were counted, and the body lengths of 15-25 individuals of each taxon were measured to the nearest 0.05 mm with an optical micrometer. The enumerated taxonomic groups were *Daphnia pulicaria*, Calanoid copepods, Cyclopoid copepods, nauplii, and 'other Cladocera', which included *Diaphanosoma, Bosmina,* and *Leptodora*. For samples of the entire water column (22-0 m) biomass was computed from body length with empirical regression equations (Malley et al. 1989). Additional samples of *D. pulicaria* were collected with a coarser filter (800 μm) that allowed smaller-bodied taxa to pass. The clutch size and body length (tip of the head to the base of body excluding the tail spine) of *Daphnia* in these samples (usually 80-100 individuals) were recorded. The minimum body length of *D. pulicaria* captured using the 800 μm filter was 1.25 mm. The smallest individual with a clutch of eggs was

1.30 mm long, so the coarser filter effectively sampled the adult *D. pulicaria*, while the 130 μm filter captured *Daphnia* of all sizes.

Phytoplankton Sampling

Water samples for chlorophyll a analyses were collected from the epilimnion with a Van Dorn water sampler on each sampling date. Samples were filtered through a glass fiber filter and pigments were extracted with methanol (Holm-Hansen and Reiman 1978). If chlorophyll analyses were not done immediately, the filters were frozen for future analysis.

Trout Sampling

On eight dates (Table 3), trout or trout stomachs were obtained from anglers for gut content analyses. The stomachs were dissected and their contents were gently rinsed over a sieve (230 μm mesh) with distilled water. Stomach contents were identified, enumerated, and measured in the same manner as other zooplankton samples.

Demographic Analyses

Egg ratio analyses (Paloheimo et al. 1982) were used to determine the reproductive rate of the *Daphnia* population in each year. This information was compared to the mortality imposed by trout to assess the impact of trout predation on the dynamics of the *D. pulicaria* population within and among years.

Egg development time (D, days) was calculated from temperature (T, $^{\circ}$C) with:

$$\ln (D) = \ln(3.3956) + \ln(T) - 0.3414(\ln(T))^2 \qquad \text{(Eq. 1)}$$

(Bottrell et al. 1976; Sterner 1998). The average temperature was estimated for the population over a 24-h period. To account for the diel vertical migration, it was assumed that *Daphnia* was exposed to the mean temperature below the epilimnion (excluding depths where oxygen concentrations were < 1 mg/L) during the day, and the mean temperature of the epilimnion at night. The average temperature in these environments, adjusted for daylength, was used to calculate D. *Daphnia* birth rate (b, day^{-1}) was calculated by:

$$b = \ln (E/D + 1), \qquad \text{(Eq. 2)}$$

where E is the mean number of eggs per individual. The birth rate multiplied by population size is the reproductive rate of the population (R, # births day^{-1}).

A bioenergetics analysis of rainbow trout in another Minnesota lake (Hirsch and Negus 2000) calculated that the mean daily per capita consumption of *Daphnia* by trout was 12,000 (*Daphnia* $trout^{-1}day^{-1}$). The product of this value and the size of the trout population provided an estimate of the daily mortality of imposed by trout on *Daphnia*.

Results

Zooplankton Community

Calanoid and cyclopoid copepods, and *D. pulicaria* usually dominated the biomass of the zooplankton community (Fig. 6). In 1996 and 1997, copepods comprised most of the zooplankton in May and June, but *D. pulicaria* was most important later in the year. In contrast, *D. pulicaria* were predominant during both spring and summer in both 1998 and 1999.

Daphnia Population Dynamics and Spatial Distribution

The *D. pulicaria* population dynamics were much different in years after trout were stocked in the fall (1996-1997) than after spring stocking (1998-1999). Echograms (Fig. 7), and concentrations of *Daphnia* equivalents derived from echograms (Fig. 8) show that the *Daphnia* population was very small (< 100 m^{-3}) during spring after fall stocking. In both years, the population increased during the summer, and reached its maximum in the fall (~ 6000 m^{-3} in October 1996 and September 1997). After spring stocking, concentrations of *Daphnia* were substantially larger (~ 6400 m^{-3} in April, 1998 and ~ 450 m^{-3} in April, 1999) in early spring than they were during springs after fall stocking. Trout were stocked within a week after the April sampling dates in 1998 and 1999. In both years, the *Daphnia* population grew after the trout were added, and became most abundant during May and June (maxima of ~ 63100 m^{-3} on May 25, 1998 and ~ 12000 m^{-3} on June 27,1999). Population size subsequently decreased and fell to low densities in the fall (< 300 m^{-3} in October of 1998 and 1999).

With few exceptions (11 October 1996, 21 September 1997, and 22 April 1998) the population was distributed below the epilimnion during the day (Fig. 7) and above depths where oxygen concentrations were less than 3 mg/L (Fig. 9).

Sonar Estimates of Trout Abundance

Counts of fish echoes from depths containing viable trout habitat (< 21 °C and > 5 mg/L O_2) show that trout were more abundant during the open-water season in the years of spring stocking (Table 2). In 1996 and 1997, trout abundance was moderate in early summer, but decreased to low levels by the late summer and fall (August-October). In 1998 and 1999 the size of the trout population declined from peak levels in May to minimum levels in October, but maintained substantially higher levels than in 1996-1997. Trout abundance was very low (< 164) on the three dates (11 October 1996, 21 September 1997, and 22 April 1998) that *Daphnia* was most abundant in surface waters.

Trout predation

Stomach analyses of trout show that *D. pulicaria* was their preferred prey (Table 3). The average number of *D. pulicaria* in trout stomachs ranged from 1293 to 3639. The diet of the trout was almost exclusively pelagic taxa, except on 25 May 1998, when chironomid midges and *Gammarus* were a substantial component. In addition to selectively feeding on *Daphnia*, the mean size of *Daphnia* consumed by trout was significantly larger (two sample t-tests; $p < 0.001$) than the mean size of *Daphnia* in the water column on all but one of the dates (Table 4). The average length of *Daphnia* in the trout stomachs, with all the data pooled (n = 780) was 2.10 mm, and the smallest

individual was 1.40 mm long (Fig. 10). Since the smallest individual observed with a brood was 1.30 mm long, predation by trout was apparently restricted to adult *Daphnia*.

The average adult body length (from 800 μm filter samples) typically ranged between 1.8 to 2.2 mm (grand mean +/- s.d. for all dates = 2.04 +/- 0.17 mm), with a few notable exceptions. On the last two sampling dates of 1997 (7 August, and 21 September) and the first sampling date of 1998 (22 April), when the rainbow trout population was small ($\leq$ 730), the mean adult *Daphnia* body size was significantly larger than the grand mean (Fig. 11).

Effect of Daphnia on Algae and Water Transparency

A regression of the mean epilimnetic chlorophyll a concentrations versus mean *Daphnia* population density for May-October dates (for all years) provides evidence that *Daphnia* grazing had a significant ($p = 0.0001$, $R^2 = 0.59$) negative effect on algal abundance (Fig.12).

In addition, secchi transparency and light transmission data suggest that the magnitude of the *Daphnia* population affected water clarity. In the years that trout were stocked in the fall (1996 and 1997), secchi transparency was low (2.8 - 5.0 m) between May and August, and highest in the fall (7.0 m on 11 October, 1996 and 5.2 m on 22 September, 1997) when *Daphnia* was most abundant (Fig. 13). Transmissometer measurements further reveal the effect of *Daphnia* grazing on water clarity. Contour plots (Fig. 14) for 1996 and 1997 show that transparency was highest in depth increments where *Daphnia* was most abundant (Figs. 14 & 7). Light transmission exceeded 90% in the metalimnion after 1 August 1996, and in surface waters in October when *Daphnia* were found in surface waters during the day (Fig. 7). Light transmission was also high (>

90%) in the metalimnion in August and September of 1997 (Fig. 14) when the density of the *Daphnia* population was high (Fig. 8).

In 1998 and 1999, when trout were stocked in the spring, the secchi transparency was considerably greater than in the previous two years (Fig. 13). In 1998, the maximum water clarity (9.2 m) occurred on 25 May when the *Daphnia* population density was at its maximum (Fig. 8). The secchi depth declined to 5 m by late July, and remained fairly constant about that value for the remaining sampling dates in 1998. As in 1998, the maximum secchi depth in 1999 (8.1 m on 27 June) coincided with the maximum density of the *Daphnia* population (Fig. 8). The secchi transparency subsequently decreased and fluctuated between 4.5 and 6 m for the remainder of the 1999 sampling dates.

Demographic Analysis

The results presented thus far show that *Daphnia* were more abundant early (April-July) in the years after spring stocking than they were after stocking in the fall. High concentrations of *Daphnia* probably caused lower chlorophyll levels and greater transparency in these years. The following demographic analysis evaluates the effect of trout predation on the *Daphnia* population, and provides a mechanistic explanation for these findings.

The reproductive rate of *Daphnia* (R, # day^{-1}) depends upon the population size (N, # of individuals), the fecundity of the population (E, # eggs $individual^{-1}$), and the development time (D, days) of parthenogenetic eggs. *Daphnia* mortality (# day^{-1}) due to trout predation was assumed to equal the product of the trout population size and the per capita consumption of *Daphnia* (12,000 day^{-1}, from Hirsch & Negus 2000).

Small population sizes early in 1996 and 1997 resulted in relatively low population birth rates despite high levels of fecundity (Table 5). Losses due to trout predation nearly balanced reproduction by *Daphnia* in May of 1997 (Fig. 15). It was not possible to calculate the reproductive rate for the 31 May 1996 sampling date because no adult *Daphnia* were obtained in net samples of the water column that were used to assess clutch size. The scarcity of adult *Daphnia* on that date, however, suggests that trout predation controlled the growth of the *Daphnia* population in May of 1996 as well. Reproduction began to exceed losses due to trout predation in June of 1996 and 1997, and the gap between these rates widened thereafter as trout became scarce.

The patterns of reproduction and mortality in 1998 and 1999 are similar to each other (Fig. 15), and provide an explanation for the large population sizes of *Daphnia* that were observed in the early part of the open water season (April-June) of these two years. The over-wintering *Daphnia* population present on 22 April 1998 was large, and adults had large brood sizes (7.07 +/- 0.57), resulting in a very high reproductive rate for the population (Table 5). This high reproductive rate enabled the population to grow by an order of magnitude over the next month (Fig. 8) in spite of the addition of 14,500 trout on 23 April 1998. Intense grazing pressure by *Daphnia* in May and June probably caused the exceptionally clear water in these months (Fig. 13). The population size decreased later in the year as a result of lower clutch sizes (Table 5) and increased the importance of trout predation (Fig 15).

The number of *Daphnia* that survived the winter in 1999 was not as large as in 1998 (Fig. 8). Estimates of trout abundance suggest that trout were substantially more abundant over the winter of 1998-1999 than they were in the winter of 1997-1998. Trout

predation over the winter is the likely explanation for the smaller *Daphnia* population in April of 1999. Because the population present at ice-out in 1999 was smaller than the population 1998, the reproductive rate was lower (Table 5, Fig. 15) and the population did not grow as explosively as it did in 1999 (Fig. 8). The population size (Fig. 8) and water clarity (Fig. 13) peaked roughly a month later in 1999 than in 1998.

Discussion

The contrast in the *Daphnia* population dynamics and the water quality of the lake between the autumn and spring stocking years is striking. When trout were stocked in the fall, predation by trout on *Daphnia* over the winter prohibited the development of a "seed" population capable of exploiting the spring phytoplankton bloom. In contrast, when trout were stocked in the spring, *Daphnia* were relatively free from predation during the winter and many survived into the spring. The large spring *Daphnia* populations coupled with high spring fecundity (Table 5) allowed the *Daphnia* populations to grow despite intense predation by the newly-stocked trout. Grazing by these abundant *Daphnia* caused much clearer water in spring and early summer than in years when trout were stocked in the fall (Fig. 13).

Data from the fall stocking years provide a clear example of predation by zooplanktivorous fish causing a trophic cascade that reduced the abundance of large *Daphnia*, increased phyoplankton abundance, and decreased water clarity. However, the interpretation of these data may have been cryptic without analyzing the demography of the *Daphnia* population. The sonar system was integral in this analysis because it provided both a more accurate measure of the size of the *Daphnia* population than could have been determined solely with conventional sampling, and information about the size of the trout population.

Another insight made evident by the acoustic data was that on dates (October 1996, September 1997, April 1998) when trout were especially scarce (Table 2) the *Daphnia* population was more shallowly distributed (Fig. 7) than on other dates. Laboratory studies have shown that chemicals (kairomones) exuded from fish induce

negative phototaxis (movement away from light) in *Daphnia* (e.g., De Meester 1993). Kairomone concentration is a cue that zooplankton rely on to assess predation risk and to mediate their diel vertical migration behavior. Loose and Dawidowicz (1994) showed that *Daphnia* became less negatively phototactic when kairomone concentrations dropped below a certain threshold level (indicating low predation risk). They concluded that *Daphnia* adjusted their daytime depths to shallower and warmer waters when predation risk was low to increase their development and reproduction rates. The observations of a shallow distribution of *Daphnia* in Long Lake when trout abundance was low are consistent with findings of these studies. While the shift in spatial distribution could be entirely an induced response, other evidence suggests that a change in the genetic composition of the population after the switch to spring stocking (Chapter 3) may have influenced the population's spatial distribution. In the late summer and fall of 1997 and in April of 1998, the dominant clone in the population was most often found in shallow water. After the advent of spring stocking and intensified trout predation, the shallow water clone decreased in abundance and was replaced by a deep-water clone.

Natural versus Managed Fish Populations

A fundamental difference between managed and natural fish populations is the nature of recruitment. In natural populations, abundance is highly variable between years because of the variability in year class success. For example, a strong year class of cisco (*Coregonus artedi*) in the late 1970's in Lake Mendota resulted in high levels of planktivory over the next ten years (Rudstam et al. 1993). The high planktivory was

associated with smaller *Daphnia pulicaria* populations and a less pronounced spring clear-water phase.

For managed fish populations that do not reproduce in the lake to which they are added (e.g., rainbow trout in Long Lake), recruitment is artificial. Population size is therefore determined by the fixed number of fish that are stocked, and losses incurred by starvation, disease, or predation (including fishing).

Studies of other lakes in Minnesota that are managed for rainbow trout (Wang et al. 1996); (Hirsch and Negus 2000) have concluded that trout predation does not significantly affect the abundance of *Daphnia* because either 1) the trout are not obligate predators of *Daphnia* throughout the year and switch their diet to prey from near-shore benthic regions (chironomid larvae and pupae) in mid-summer (Wang et al. 1996), or 2) the stocking level is low enough that mortality caused by trout predation is insignificant to the *Daphnia* population (Hirsch and Negus 2000).

In Long Lake, trout are essentially obligate predators of *Daphnia* (Table 3). The morphometry of the basin of Long Lake promotes this tight predator-prey coupling because the small littoral zone of the lake restricts the trout to pelagic waters once the lake stratifies in the summer. For this reason, when trout are abundant (Table 2) they can significantly affect the demography of the *Daphnia* population (Figure 15). Because the trout are size-selective predators (only consumed *Daphnia* > 1.4 mm in length), they both directly reduce population size and inhibit future increases by removing adult-sized (> 1.3 mm) individuals that have the potential to reproduce. However, the results of this work also illustrate that mortality resulting from trout predation is essentially irrelevant to the *Daphnia* population once it has reproductive inertia (Fig. 15).

From a management perspective, the switch from autumn to spring stocking was a virtual panacea. Not only did the sizeable over-wintering populations of *Daphnia* in 1998 and 1999 enable clearwater phases (Lampert et al. 1986; Luecke et al. 1990) that were suppressed in autumn stocking years, but they also provided abundant food for the trout and resulted in better survival (Table 2). In lakes in which the trout are largely restricted to foraging in the pelagic zone (e.g., Long Lake), the maintenance of a *Daphnia* population is as critical as the environmental habitat requirements (i.e., cold (< 21 °C), well-oxygenated (> 5 mg/L) water) to the survival of the stocked fish. Our results suggest that synchronizing trout stocking with the springtime population growth phase of *Daphnia* will maximize the per capita food supply of trout while enabling the *Daphnia* population to grow, and that the timing of biomanipulations is as important as the level at which fish are stocked.

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Tables

Table 1. Rainbow trout stocking schedule.

YEAR	TIME OF STOCKING	OPEN-WATER SEASON AFFECTED	STOCKING LEVEL		SIZE
			#	# ha^{-1}	
1995	Autumn	1996	14,500	243	Fingerling
1996	Autumn	1997	14,500	243	Fingerling
1997	NA	NA	0	0	NA
1998	Spring	1998	14,500	243	Yearling
1999	Spring	1999	14,500	243	Yearling

Table 2. Estimates of trout abundance obtained by counting fish echoes on echograms from depths that contained viable trout habitat (see Methods and Fig. 4). Counts from the long-axis transect for 25 May 1998 were not made because fish echoes could not be clearly discerned from zooplankton backscattering. For that date, the value for the zig-zag counts was used to estimate trout abundance. Dashes in the 'Zig-Zag Count' column indicate that those data were not collected. When the standardized count value was equal to or less than the 22 April 1998 value, trout were considered to be present at 'trace' levels.

DATE	LONG-AXIS COUNT	ZIG-ZAG COUNT	STANDARDIZED COUNT VALUE	ESTIMATED TROUT ABUNDANCE
6/12/1996	98	-	125	7040
7/18/1996	115	-	147	8410
8/1/1996	38	-	48.6	2190
8/26/1996	2	-	2.6	Trace
10/11/1996	12	-	15.4	80
5/17/1997	122	161	156	8980
6/16/1997	115	-	147	8410
7/7/1997	36	45	46.1	2020
8/7/1997	20	-	25.6	730
9/21/1997	13	-	16.6	160
4/22/1998	11	-	14.1	Trace
5/25/1998	-	208	-	12680
6/27/1998	132	179	169	9790
7/25/1998	110	152	141	8010
8/22/1998	77	82	98.6	5340
10/24/1998	36	36	46.1	2020
4/24/1999	48	86	61.4	2990
5/31/1999	246	292	315	19000
7/10/1999	121	204	155	8900
8/19/1999	87	97	111	6150
10/2/1999	44	43	56.3	2670

Table 3. Stomach contents of rainbow trout. Values are the mean number (+/- s.e.) of each taxon per trout stomach. Values for *D. pulicaria*, Calanoid and Cyclopoid copepods, and *Gammarus* were calculated by subsampling. All of the larger invertebrates, *Leptodora*, Chironomids, and *Chaoborus*, were individually picked from the samples and counted.

DATE	# OF TROUT	*D. PULICARIA*		CALANOID		CYCLOPOID		*LEPTODORA*		GAMMARUS		CHIRONOMID		*CHAOBORUS*	
		Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
07/07/1997	1	1490	NA	70	NA	0	NA	30	NA	0	NA	0	NA	10	NA
05/25/1998	4	3059	1418	0	-	0	-	0	-	5.0	5.0	61.3	21.0	0	-
06/27/1998	7	2101	655	20.0	10.2	0	-	0	-	0	-	0	-	0	-
07/25/1998	5	2139	410	9.1	7.8	5.3	5.3	0	-	0	-	1.1	1.1	0	-
08/22/1998	3	3463	1362	0	-	0	-	5.0	5.0	0	-	1.7	0.9	0	-
05/31/1999	2	2983	83	16.7	16.7	0	-	0	-	0	-	0	-	0	-
06/27/1999	3	1293	272	13.3	13.3	0	-	0	-	0	-	0	-	0	-
07/10/1999	2	3639	615	27.5	7.5	0	-	0	-	0	-	0	-	0	-

Table 4. T-tests comparing the mean size (length, mm) of *D. pulicaria* in the water column versus those in the stomachs of rainbow trout. *Daphnia* consumed by trout were significantly larger than those in the water column on all but one date (22 August, 1998) when trout stomach contents were evaluated.

DATE	WATER COLUMN (MM)	S.E. (MM)	N	TROUT STOMACH (MM)	S.E. (MM)	N	T STATISTIC	DF	P-VALUE
7/7/1997	1.47	0.11	15	1.95	0.02	94	6.21	107	< 0.001
5/25/1998	1.78	0.10	15	2.25	0.03	94	5.95	107	< 0.001
6/27/1998	1.86	0.08	20	2.12	0.02	139	4.02	157	< 0.001
7/25/1998	1.64	0.10	20	2.14	0.02	100	8.13	118	< 0.001
8/22/1998	1.91	0.06	20	1.96	0.02	71	1.31	89	0.195
5/31/1999	1.51	0.12	25	2.20	0.02	107	9.37	130	< 0.001
6/27/1999	1.44	0.07	25	2.09	0.02	81	12.8	104	< 0.001
7/10/1999	1.51	0.09	25	2.08	0.02	94	10.0	117	< 0.001

Table 5. Demographic parameters for the adult *Daphnia* population from 1996-1999. Parameters include: estimates of duration of development (D) of eggs and embryos is based on temperature; mean numbers of eggs and embryos (E); instantaneous birth rate (b); total size of the population in the lake (N) in terms of *Daphnia* equivalents; and the reproductive rate of the population (R). For a description of the calculation of these demographic parameters see the methods section.

Date	Mean Temp Experienced (°C)	D (days)	E (mean +/- s.e.)	E/D (+/- s.e.)	b (day^{-1}) (+/- s.e.)	N (10^{10} *Daphnia* equivalents) (+/- s.e.)	R (10^{10} # day^{-1}) (+/- s.e.)
6/12/1996	11.4	5.1	5.51 +/- 0.71	1.08 +/- 0.14	0.79 +/- 0.06	0.005 +/- 0.001	0.037 +/- 0.0008
6/27/1996	11.3	5.2	1.71 +/- 0.37	0.33 +/- 0.07	0.34 +/- 0.05	0.763 +/- 0.263	0.218 +/- 0.014
7/18/1996	12.5	4.8	4.60 +/- 0.42	0.96 +/- 0.09	0.72 +/- 0.04	4.55 +/- 1.45	3.06 +/- 0.063
8/1/1996	13.3	4.6	2.43 +/- 0.24	0.53 +/- 0.05	0.46 +/- 0.03	3.45 +/- 0.752	1.46 +/- 0.025
8/26/1996	16.2	3.9	0.18 +/- 0.06	0.05 +/- 0.02	0.06 +/- 0.01	1.63 +/- 0.315	0.074 +/- 0.0045
10/11/1996	10.5	5.4	0.19 +/- 0.07	0.04 +/- 0.01	0.05 +/- 0.01	4.54 +/- 1.73	0.157 +/- 0.021
5/17/1997	6.7	6.6	4.82 +/- 1.15	0.73 +/- 0.17	0.64 +/- 0.10	0.029 +/- 0.016	0.016 +/- 0.0015
6/16/1997	12.0	5.0	9.57 +/- 0.64	1.93 +/- 0.13	1.12 +/- 0.04	0.171 +/- 0.093	0.184 +/- 0.0040
7/7/1997	11.9	5.0	3.72 +/- 0.55	0.75 +/- 0.11	0.62 +/- 0.06	0.263 +/- 0.073	0.147 +/- 0.0044
8/7/1997	16.0	3.9	8.69 +/- 0.54	2.21 +/- 0.14	1.21 +/- 0.04	1.64 +/- 0.668	1.91 +/- 0.028
9/26/1997	14.3	5.4	0.24 +/- 0.06	0.06 +/- 0.01	0.07 +/- 0.01	4.48 +/- 0.577	0.240 +/- 0.0076
4/22/1998	5.9	6.8	7.07 +/- 0.57	1.03 +/- 0.08	0.75 +/- 0.04	4.85 +/- 1.30	3.44 +/- 0.052
5.25/1998	11.2	5.2	0.41 +/- 0.12	0.08 +/- 0.02	0.10 +/- 0.02	48.1 +/- 8.81	3.62 +/- 0.190
6/27/1998	13.3	4.6	0.10 +/- 0.04	0.02 +/- 0.01	0.03 +/- 0.01	2.95 +/- 1.08	0.065 +/- 0.0092
7/25/1998	14.6	4.3	0.14 +/- 0.05	0.03 +/- 0.01	0.04 +/- 0.01	0.263 +/- 0.094	0.0085 +/- 0.0011
8/22/1998	15.6	4.0	0.31 +/- 0.07	0.08 +/- 0.02	0.09 +/- 0.02	1.29 +/- 0.404	0.094 +/- 0.0067
10/24/1998	10.3	5.5	2.91 +/- 0.39	0.53 +/- 0.07	0.47 +/- 0.05	0.177 +/- 0.063	0.076 +/- 0.029
4/24/1999	4.7	7.0	4.54 +/- 0.38	0.64 +/- 0.05	0.53 +/- 0.03	0.346 +/- 0.305	0.172 +/- 0.098
5/31/1999	10.3	5.5	6.05 +/- 0.44	1.11 +/- 0.08	0.78 +/- 0.04	2.64 +/- 1.14	1.97 +/- 0.043
6/27/1999	12.6	4.8	0.11 +/- 0.04	0.02 +/- 0.01	0.03 +/- 0.01	9.12 +/- 2.65	0.198 +/- 0.022
7/10/1999	12.5	4.8	0.03 +/- 0.02	0.01 +/- 0.00	0.01 +/- 0.00	1.00 +/- 0.279	0.0071 +/- 0.0011
8/19/1999	14.9	4.2	0.74 +/- 0.17	0.18 +/- 0.04	0.20 +/- 0.03	0.466 +/- 0.213	0.076 +/- 0.0072
10/2/1999	11.7	5.0	0.12 +/- 0.05	0.02 +/- 0.01	0.03 +/- 0.01	0.166 +/- 0.144	0.0038 +/- 0.0013

Figure legends

Figure 1. Bathymetric map of Long Lake (adapted with permission from Ross et al., 1996).

Figure 2. Map of Long Lake showing the paths for the sonar transects (solid and dotted lines) and the sampling location (black dot). The solid line along the long-axis of the lake indicates the path for the narrow-beam transducer transect. The series of dotted lines shows the path taken for the zig-zag transects using the wide-beam transducer. Locations on shore at the endpoints of the transects are labeled L1, L2,....L10.

Figure 3. Acoustic estimates of *Daphnia* abundance were calculated from sonar data from the long-axis transects. The upper panel of this figure shows a long-axis transect echogram from 27 June, 1998. The scale on the left indicates depth (m), and the color scale on the right corresponds to volume backscattering strength (dB). Distance (km) from the southeast to the northwest end of the lake is shown on the bottom. The gray area is the lake bottom. To calculate *Daphnia* abundance, the mean value of volume backscattering (dB) where *Daphnia* occurred (Boxes A & B) was translated to an estimate of mean *Daphnia* concentration (Log_{10} *Daphnia* equivalents m^{-3}) using a target strength of -120 dB (middle panel). Total population size was calculated by multiplying the density estimates by the volume of water in the relevant depth increments (lower panel).

Figure 4. Acoustic estimates of rainbow trout abundance were made by counting echo traces made by fish within the bounds of the trout habitat (delineated by horizontal black lines in the upper and lower panels). The upper panel shows a wide-beam transect between locations L5 and L6. Note the arc-shaped "trout" echoes on this echogram. Trout abundance was also estimated acoustically by filtering out the weaker backscattering (< -70 dB) from the narrow-beam transects (middle panel) and counting trout echoes. Fish traces are difficult to see when viewing the entire long-axis transect at once, but are discernable when the image is magnified (lower panel).

Figure 5. Methodology for relating the counts of fish traces from echograms to trout abundance. The slope (1.24) of the linear regression of fish counts from zig-zag transects versus long-axis transects (upper panel) was used to calculate a standardized acoustic estimate of trout abundance for all dates when a long-axis transect was performed (see Table 4 for values). Standardized estimates for 1998 were regressed against the number of days after 14,500 trout were added to the lake to relate the standardized acoustic estimates to actual trout abundance (middle panel). The y-intercept (236) of this regression is taken to represent the acoustic equivalent to 14,500 trout. The standardized estimate from 22 April 1998 (the day before trout were stocked in 1998) was assumed to equal zero trout, and the linear relationship between this value and the value equivalent to 14,500 trout was used to translate the standardized values to estimates actual trout abundance (lower panel).

Figure 6. Area plots showing the taxonomic composition of the zooplankton biomass in net tows from the water column for 1996-1999. Length-weight regression equations from Malley et al. (1989) were used to calculate biomass values.

Figure 7. Long-axis transect echograms for each year (Figs. 7a-7d) show the spatial distribution of *Daphnia*. Horizontal black lines delimit the depths at which *D. pulicaria* occurred on each date.

Figure 8. Population dynamics of *D. pulicaria* for 1996-1999. Sonar information was used to estimate population density (*Daphnia* equivalents m^{-3}) on all but four dates (open circles). On those dates, population density was estimated from conventional net sampling. Error bars represent +/- s.e.

Figure 9. Contour plots of temperature and dissolved oxygen for 1996-1999.

Figure 10. Histogram showing the distribution of body sizes of *Daphnia* present in trout stomachs (pooled data from all years).

Figure 11. Plots of the mean body length (+/- s.e.) of adult *Daphnia* on each sampling date from 1996-1999. In fall stocking years (1996-1997) the mean body size of adult *Daphnia* increased from spring to fall. Mean adult body size was largest late in 1997 (August-October) and early in 1998 (April) when trout abundance was low (see Table 2).

Figure 12. Simple linear regression of mean epilimnetic chlorophyll a (Chl a) concentration versus Log *Daphnia* concentration determined from acoustic analyses for dates from May-September in all years. The regression is significant, and shows that *Daphnia* concentration explains 59% of the variance in epilimnetic Chl a concentration.

Figure 13. Secchi depth measurements in all four years (1996-1999). Black circles indicate data we collected, and open circles are data collected by others. In 1996 supplementary data were collected by researchers from the University of Minnesota's Lake Itasca Biological Station (IBS). In 1997-1999, supplementary data were obtained from the Minnesota Department of Natural Resources (MDNR). In 1996-1997 secchi depth was relatively low early in the year and increased over the summer as the *Daphnia* population grew (Fig. 8, top two panels). In 1998-1999, water clarity was highest in late spring-early summer and decreased over the rest of the open-water season as *Daphnia* became less abundant (Fig. 8, bottom two panels). A one-way ANOVA comparing the May-September means from 1996-1999 was significant ($p < 0.0001$), and post-hoc comparisons of the means using Tukey's test show that the differences between the fall stocking (1996-1997) and spring stocking (1998-1999) years are responsible for the significance of the test. Groups marked by the same letter are not significantly different from each other ($p > 0.05$).

Figure 14. Isopleth diagrams of light transmission during 1996-1997. Light transmission increased in the water column in late summer to fall in both years as the *Daphnia* population grew. Light transmission was especially high (> 90%) at depths where *Daphnia* aggregated during the day (Figs. 7a & 7b).

Figure 15. Summary of the demographic analysis of the *D. pulicaria* population for 1996-1999 (see Table 2 for values used to calculate mortality rates due to trout predation, and Table 6 for data used to calculate production rates). In years when the *Daphnia* population was subjected to predation by trout stocked in the fall (1996-1997), mortality and reproductive rates were nearly balanced during the spring. Reproduction and mortality rates diverged thereafter in 1996-1997 as the *Daphnia* population grew and the trout population declined. In the years when trout were stocked in the spring (1998-1999), the high reproductive potential of the over-wintering *Daphnia* population enabled production by *Daphnia* to greatly exceeded trout-induced mortality during the spring. Trout-induced mortality equaled or exceeded *Daphnia* reproduction in early-mid summer of these years due to small *Daphnia* clutch sizes and better survival by trout than in 1996-1997. **Note:** open symbols indicate dates when sonar information was not obtained. On these dates, the reproductive rate of *Daphnia* (open circles) was estimated using *Daphnia* abundance data from net samples, and predation rates (open squares) were calculated using trout abundances estimated by averaging values from adjacent dates.

Figure 1.

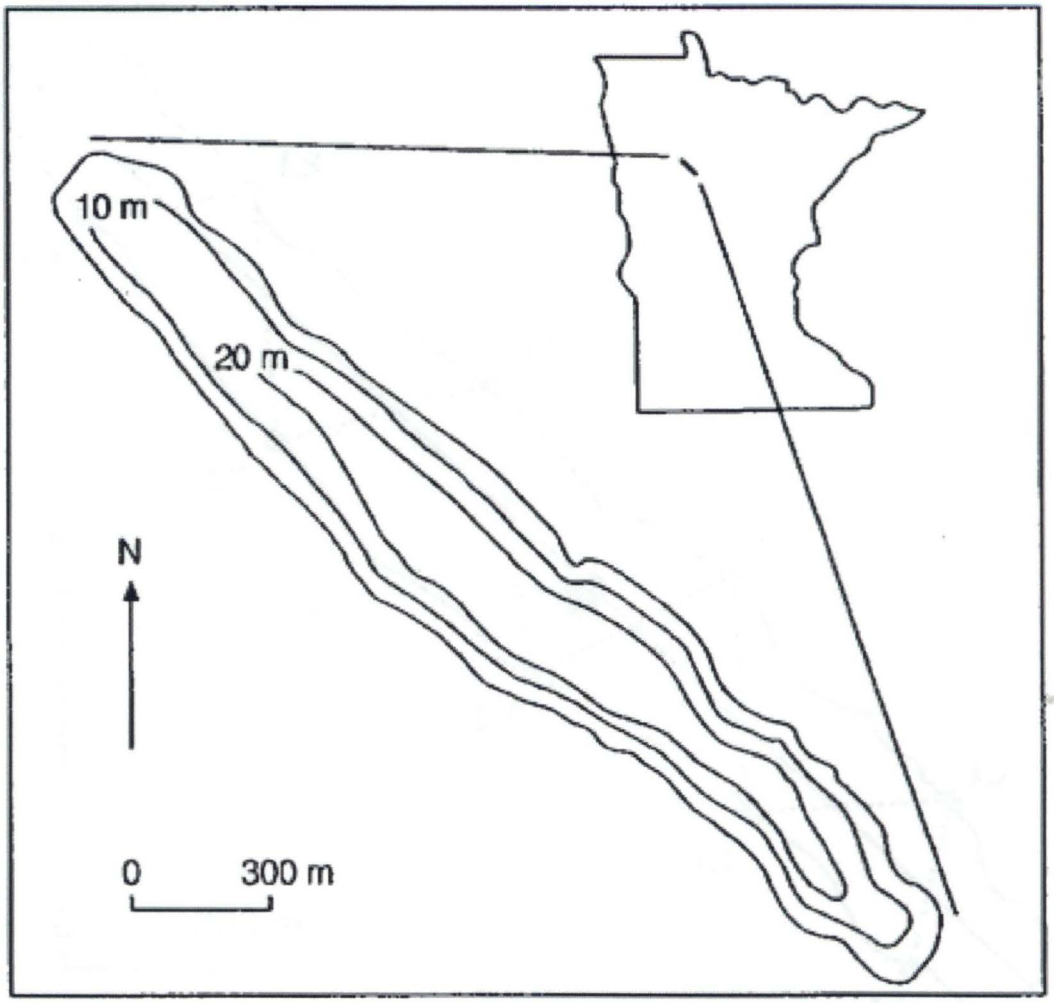


Figure 2.

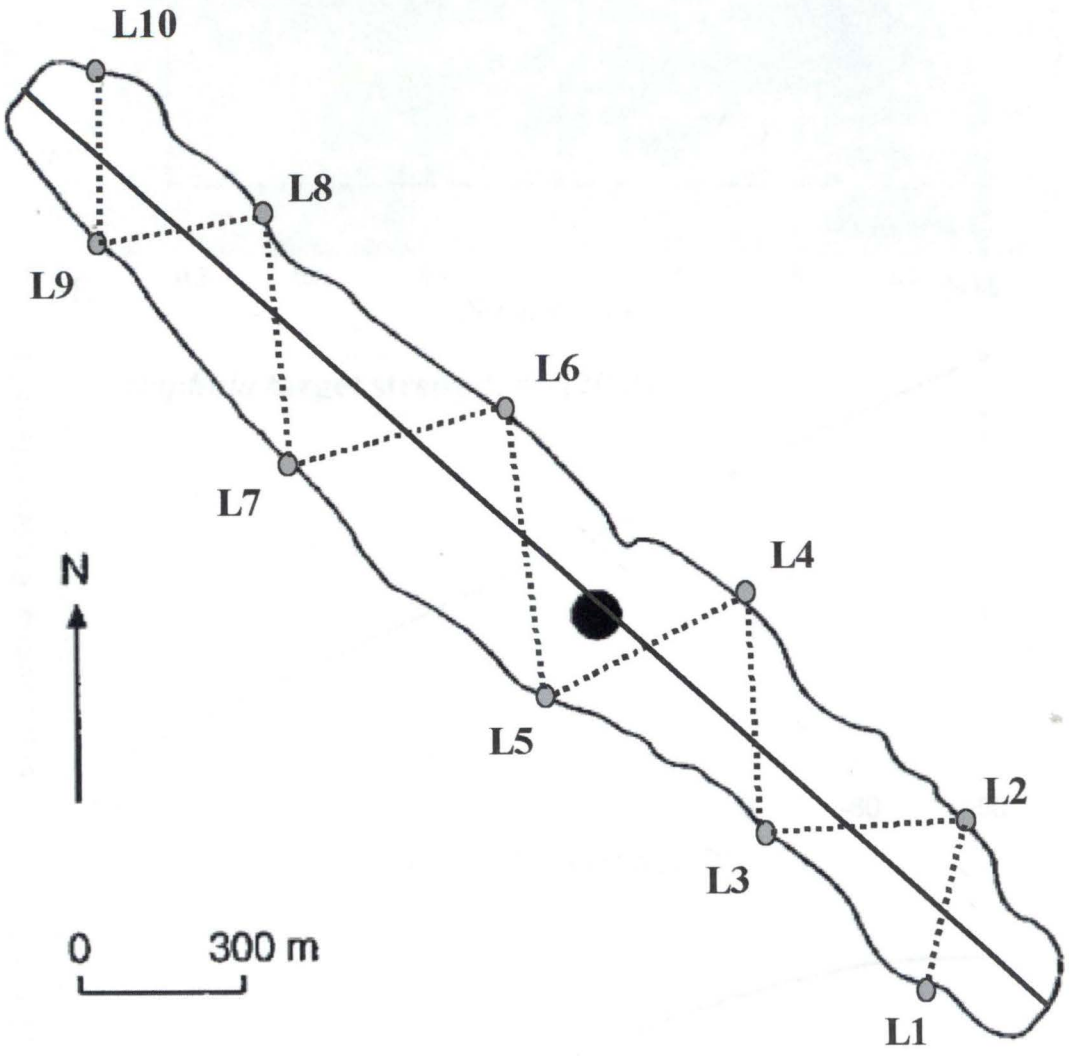
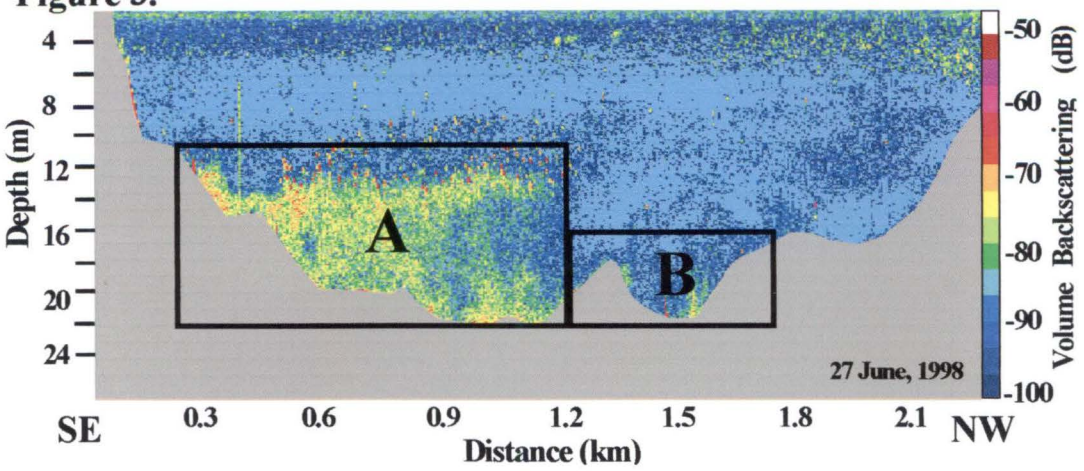
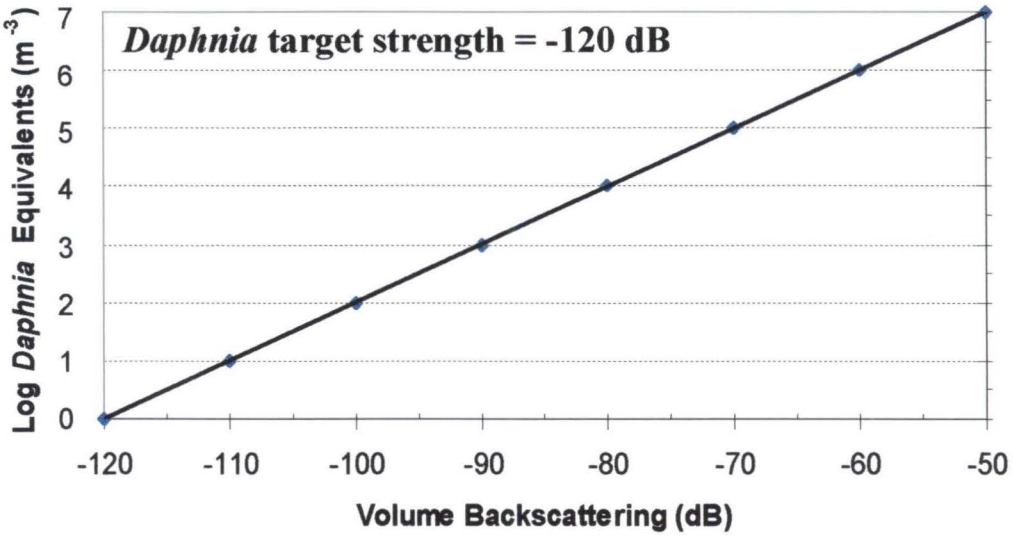


Figure 3.


Depth (m)
4
8
12
16
20
24
A
B
27 June, 1998
-50
-60
-70
-80
-90
-100
Volume Backscattering (dB)
SE
0.3
0.6
0.9
1.2
1.5
1.8
2.1
NW
Distance (km)


Daphnia target strength = -120 dB
Log Daphnia Equivalents (m-3)
0
1
2
3
4
5
6
7
-120
-110
-100
-90
-80
-70
-60
-50
Volume Backscattering (dB)

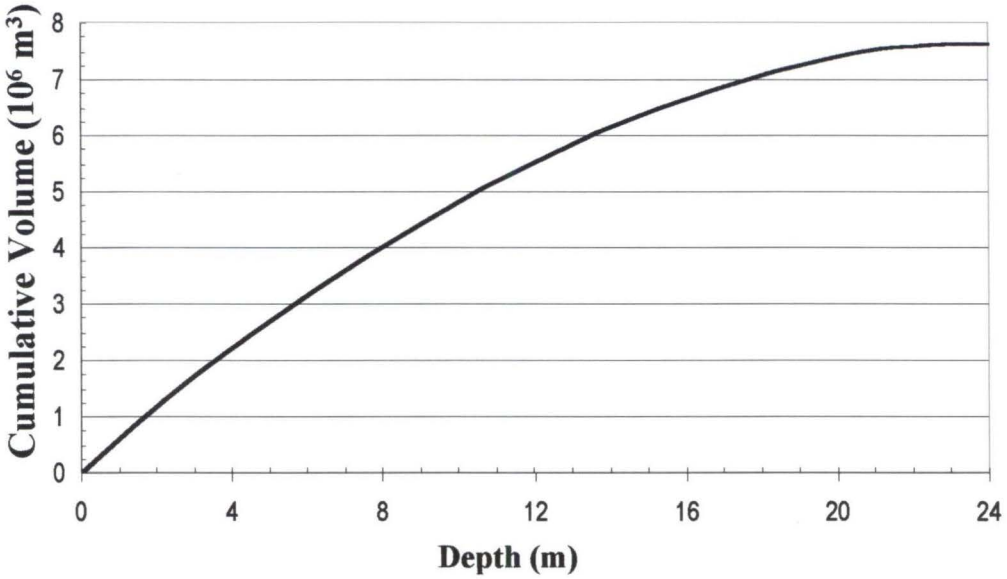

Cumulative Volume (10^6 m^3)
0
1
2
3
4
5
6
7
8
0
4
8
12
16
20
24
Depth (m)

Figure 4.

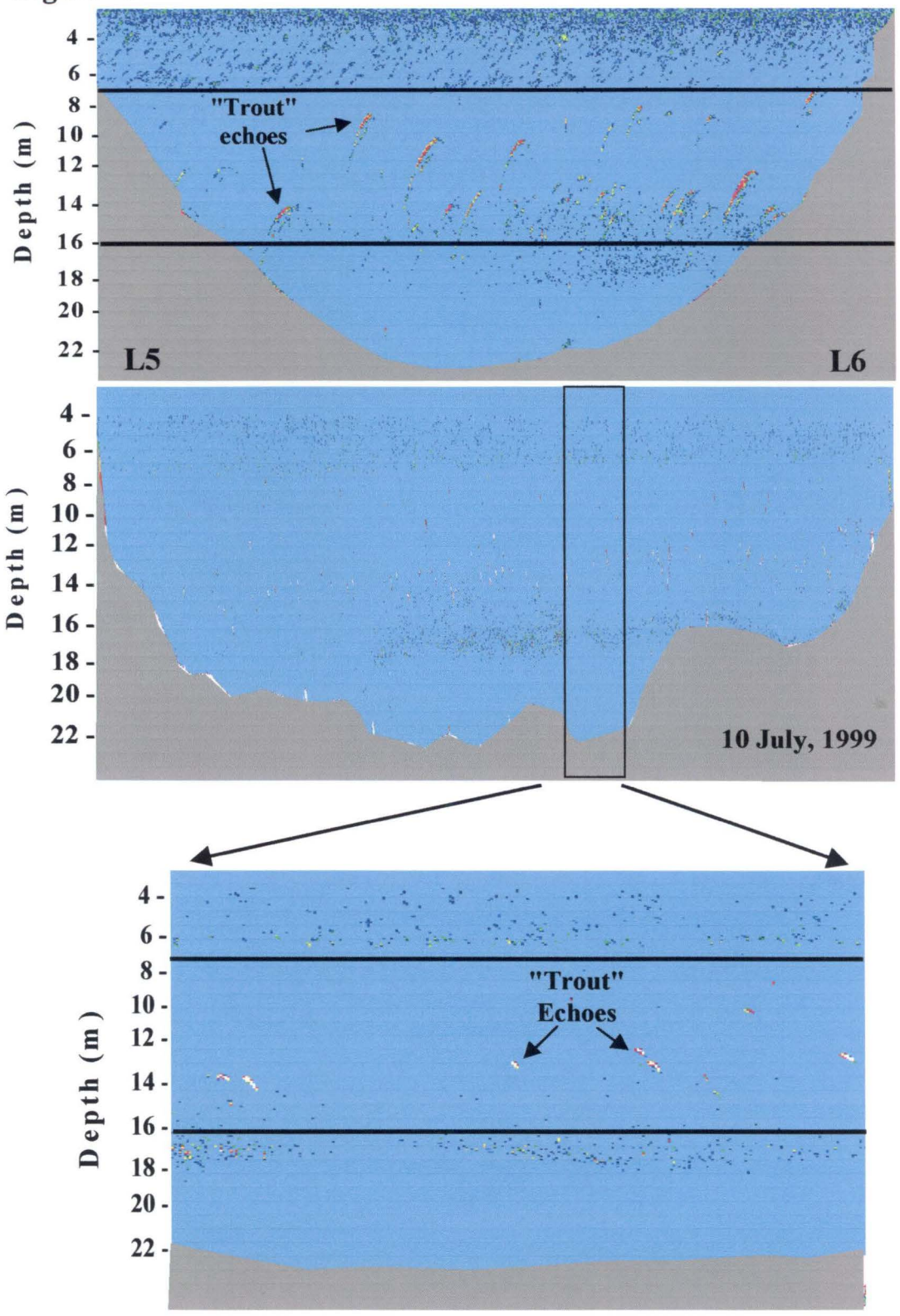
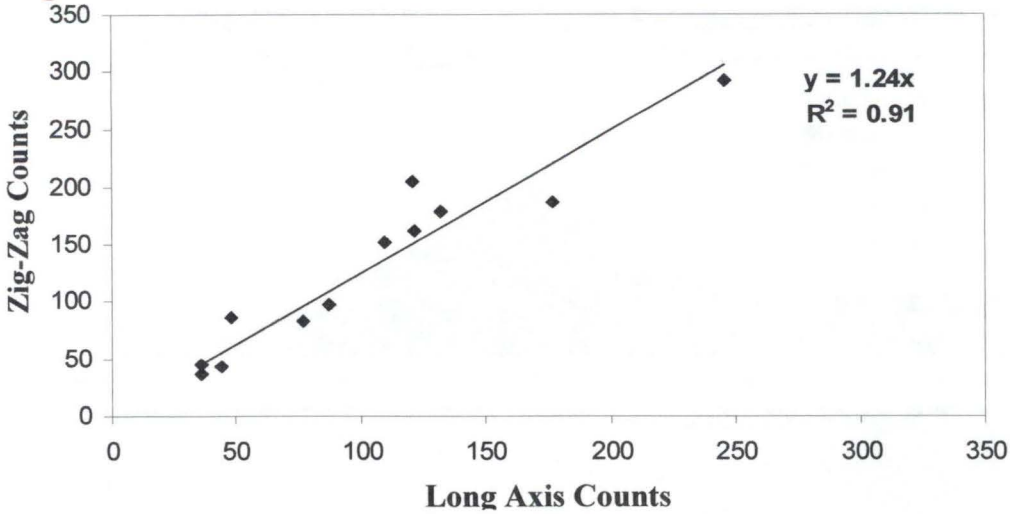
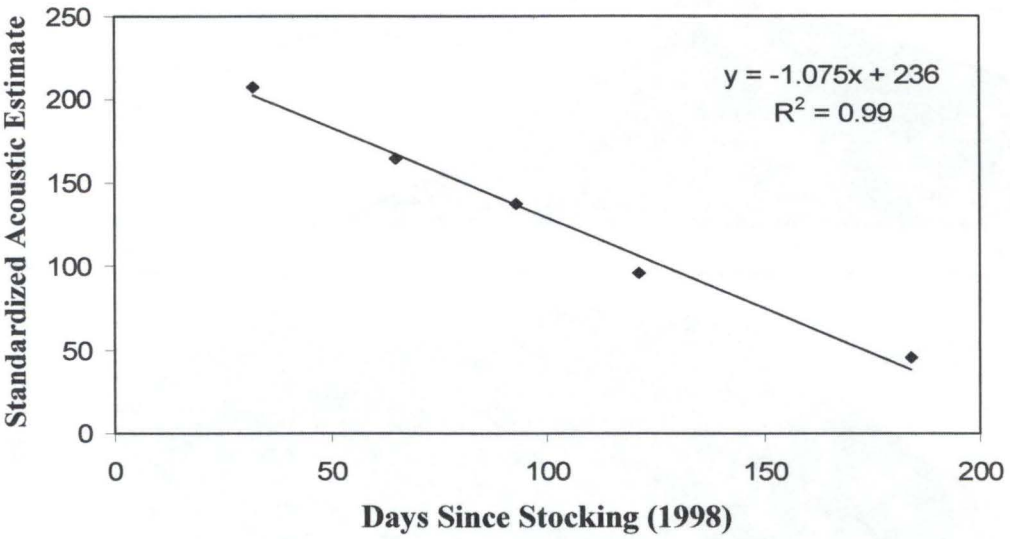

Depth (m)
4
6
8
10
12
14
16
18
20
22
"Trout" echoes
L5
L6
10 July, 1999
"Trout" Echoes

Figure 5.


y = 1.24x
R² = 0.91
Zig-Zag Counts
Long Axis Counts
0
50
100
150
200
250
300
350


y = -1.075x + 236
R² = 0.99
Standardized Acoustic Estimate
Days Since Stocking (1998)
0
50
100
150
200
250

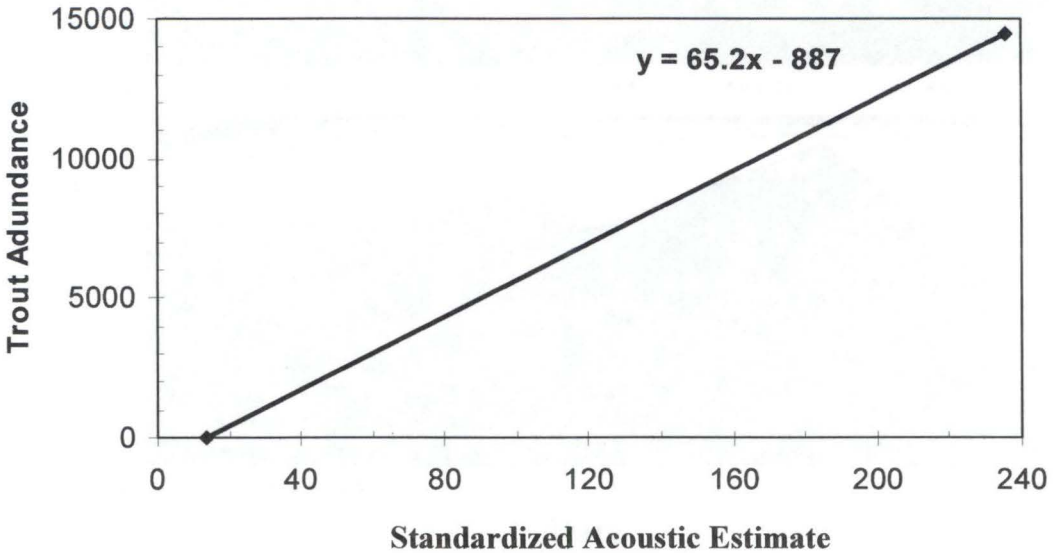

y = 65.2x - 887
Trout Adundance
Standardized Acoustic Estimate
0
5000
10000
15000
40
80
120
160
200
240

Figure 6.

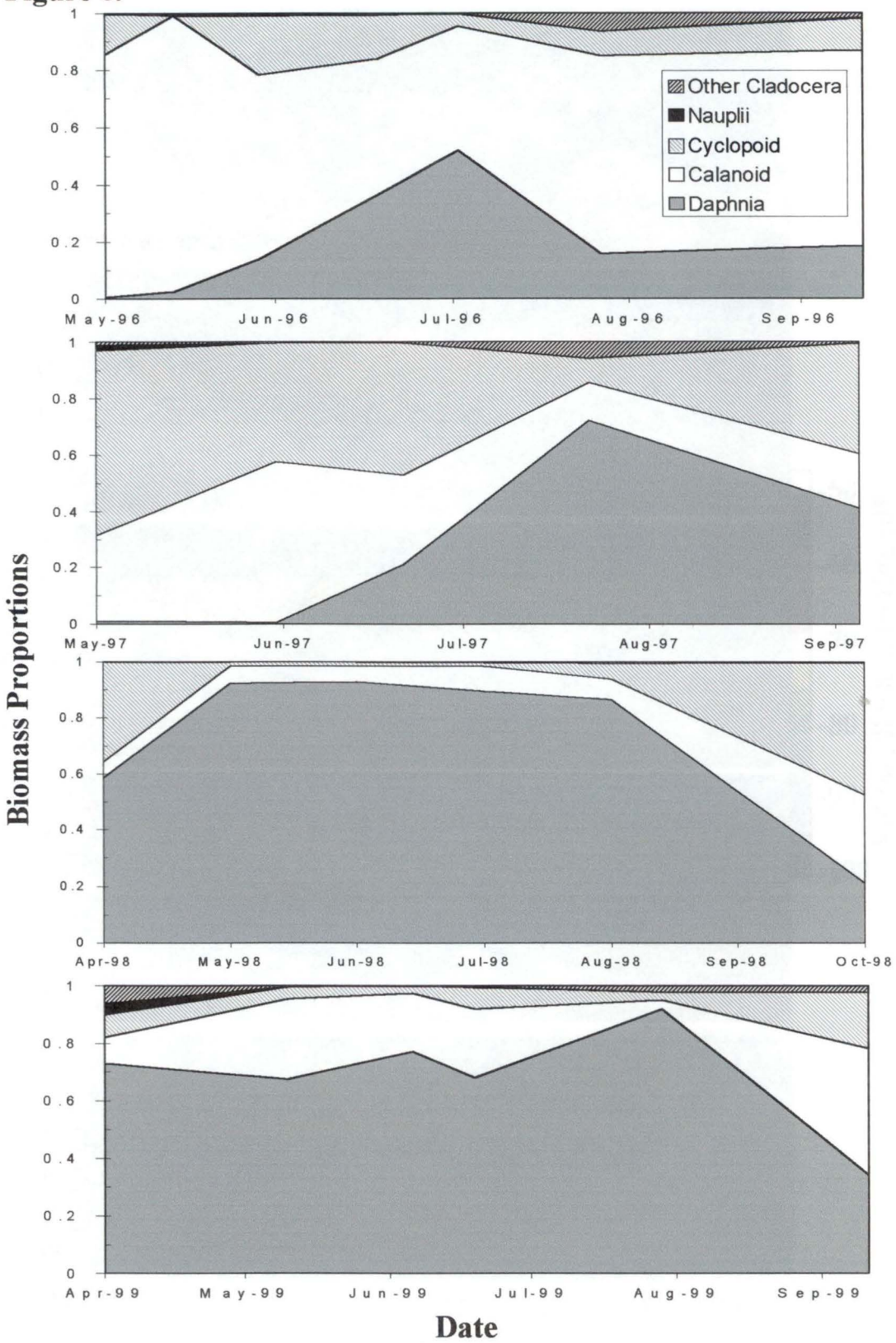

Other Cladocera
Nauplii
Cyclopoid
Calanoid
Daphnia
Biomass Proportions
1
0.8
0.6
0.4
0.2
0
May-96
Jun-96
Jul-96
Aug-96
Sep-96
May-97
Jun-97
Jul-97
Aug-97
Sep-97
Apr-98
May-98
Jun-98
Jul-98
Aug-98
Sep-98
Oct-98
Apr-99
May-99
Jun-99
Jul-99
Aug-99
Sep-99
Date

Figure 7a.

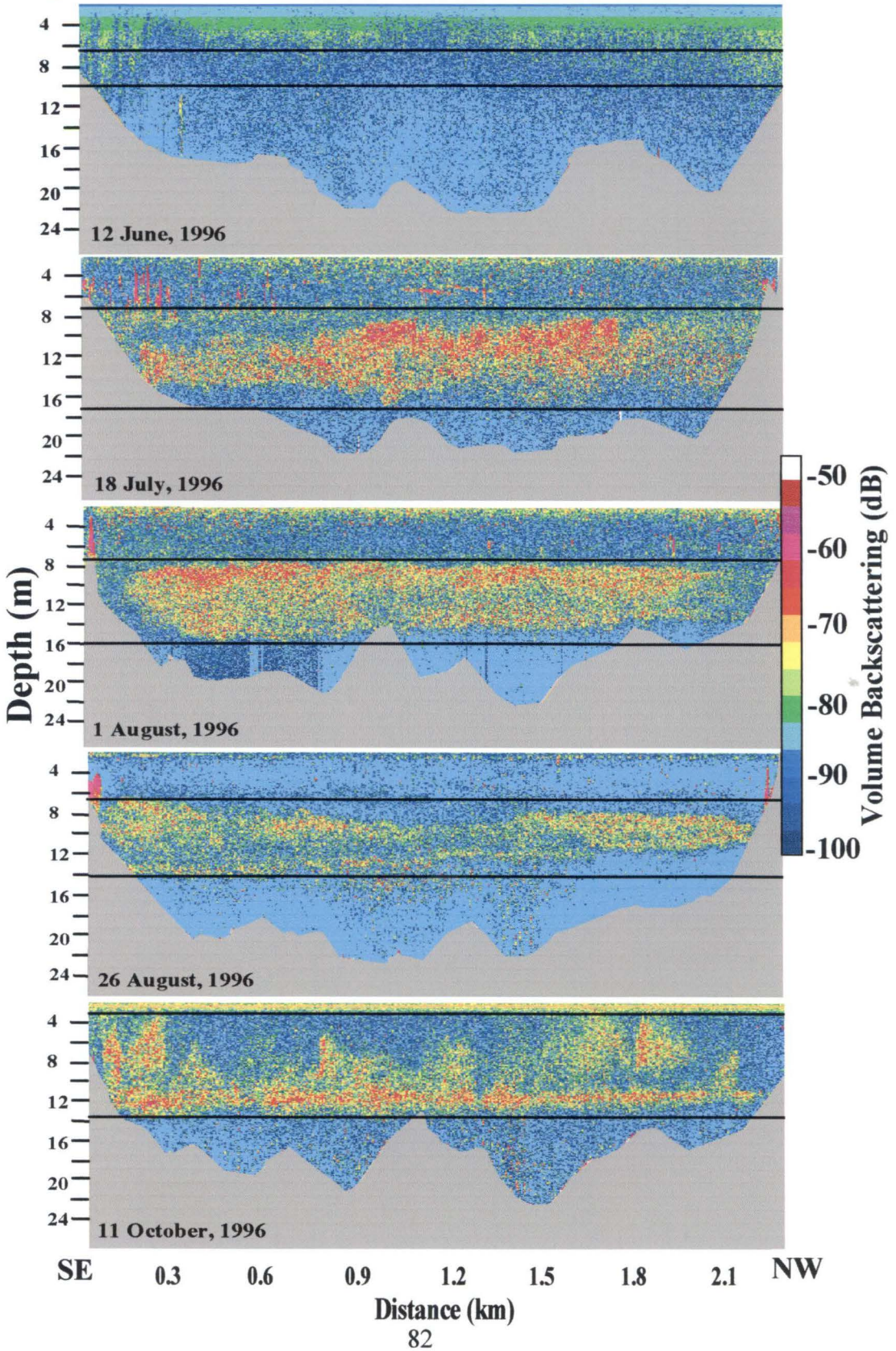

4
8
12
16
20
24
12 June, 1996
18 July, 1996
1 August, 1996
26 August, 1996
11 October, 1996
Depth (m)
-50
-60
-70
-80
-90
-100
Volume Backscattering (dB)
SE
0.3
0.6
0.9
1.2
1.5
1.8
2.1
NW
Distance (km)

Figure 7b.

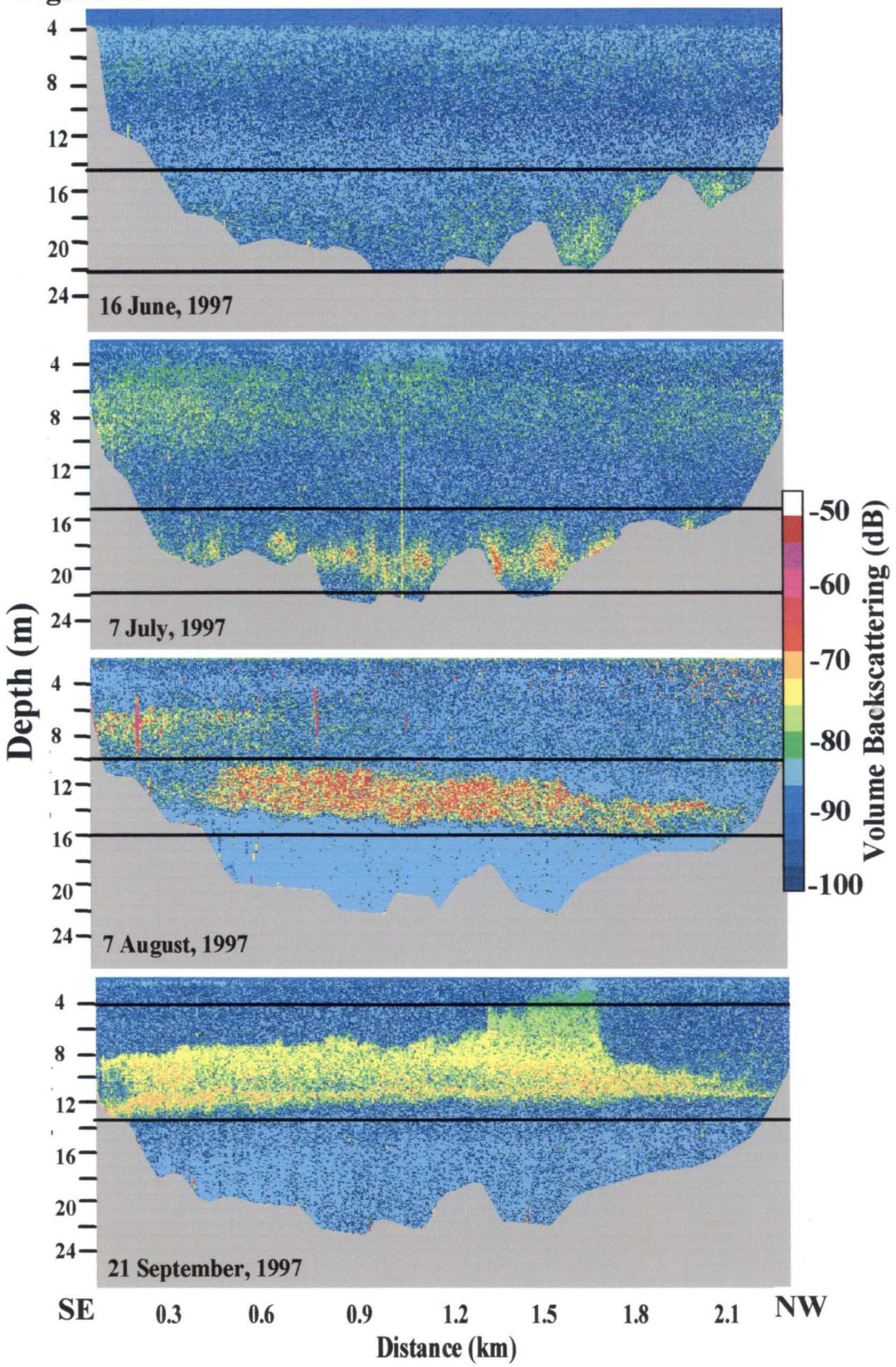

4
8
12
16
20
24
16 June, 1997
7 July, 1997
7 August, 1997
21 September, 1997
Depth (m)
-50
-60
-70
-80
-90
-100
Volume Backscattering (dB)
SE
0.3
0.6
0.9
1.2
1.5
1.8
2.1
NW
Distance (km)

Figure 7c.

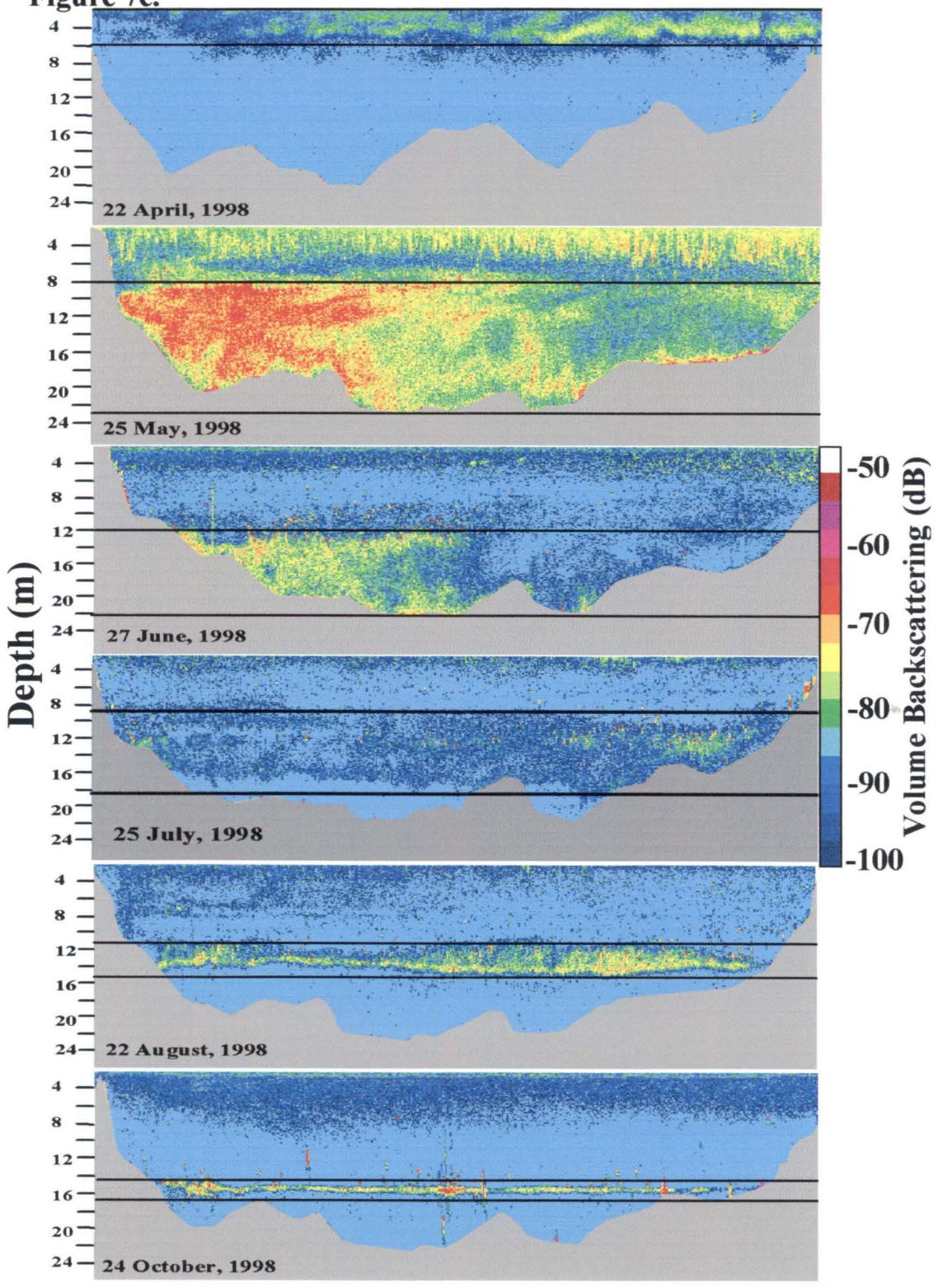

Depth (m)
4
8
12
16
20
24
22 April, 1998
25 May, 1998
27 June, 1998
25 July, 1998
22 August, 1998
24 October, 1998
-50
-60
-70
-80
-90
-100
Volume Backscattering (dB)
SE
0.3
0.6
0.9
1.2
1.5
1.8
2.1
NW
Distance (km)

Figure 7d.

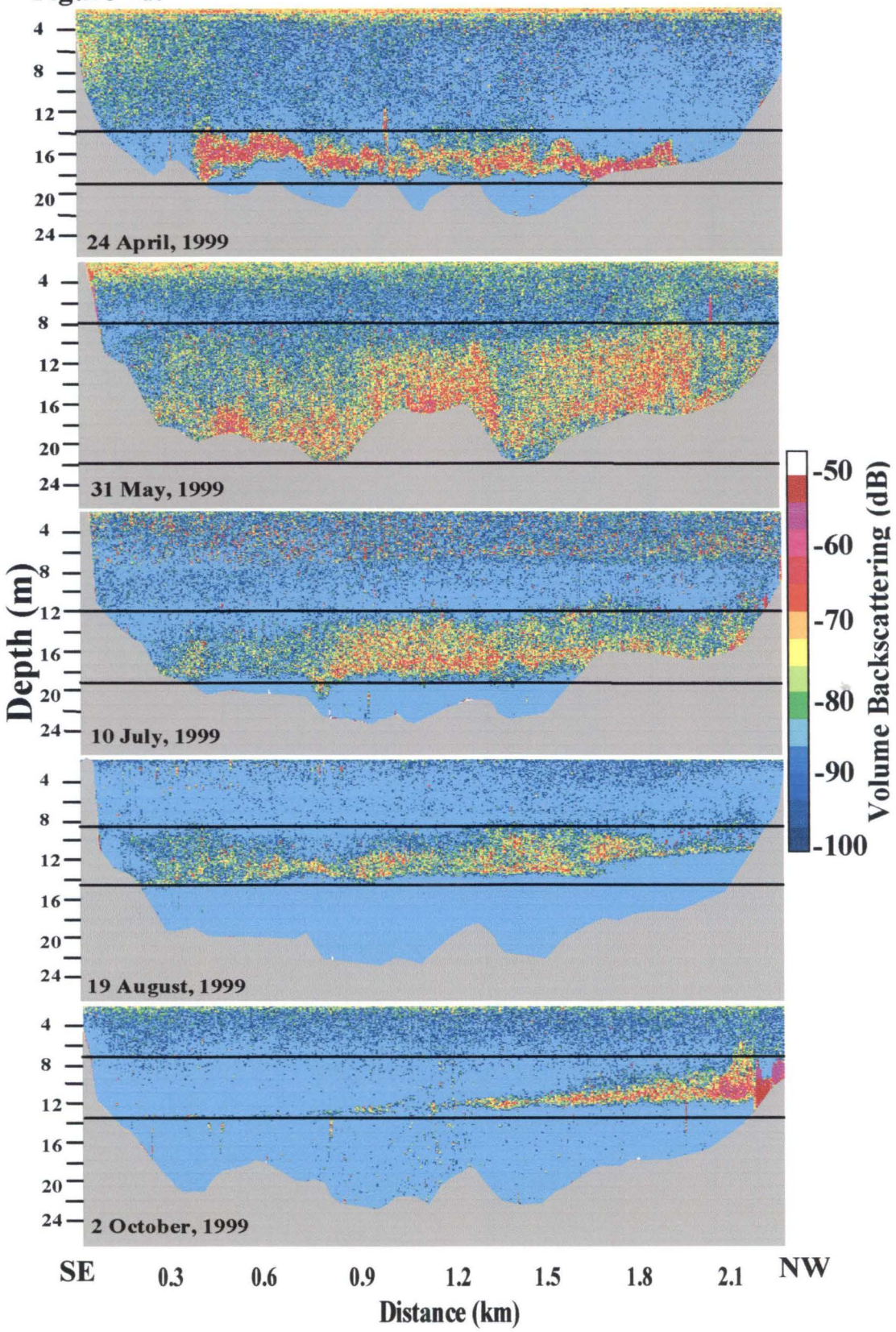

4
8
12
16
20
24
24 April, 1999
31 May, 1999
10 July, 1999
19 August, 1999
2 October, 1999
Depth (m)
-50
-60
-70
-80
-90
-100
Volume Backscattering (dB)
SE
0.3
0.6
0.9
1.2
1.5
1.8
2.1
NW
Distance (km)

Figure 8.

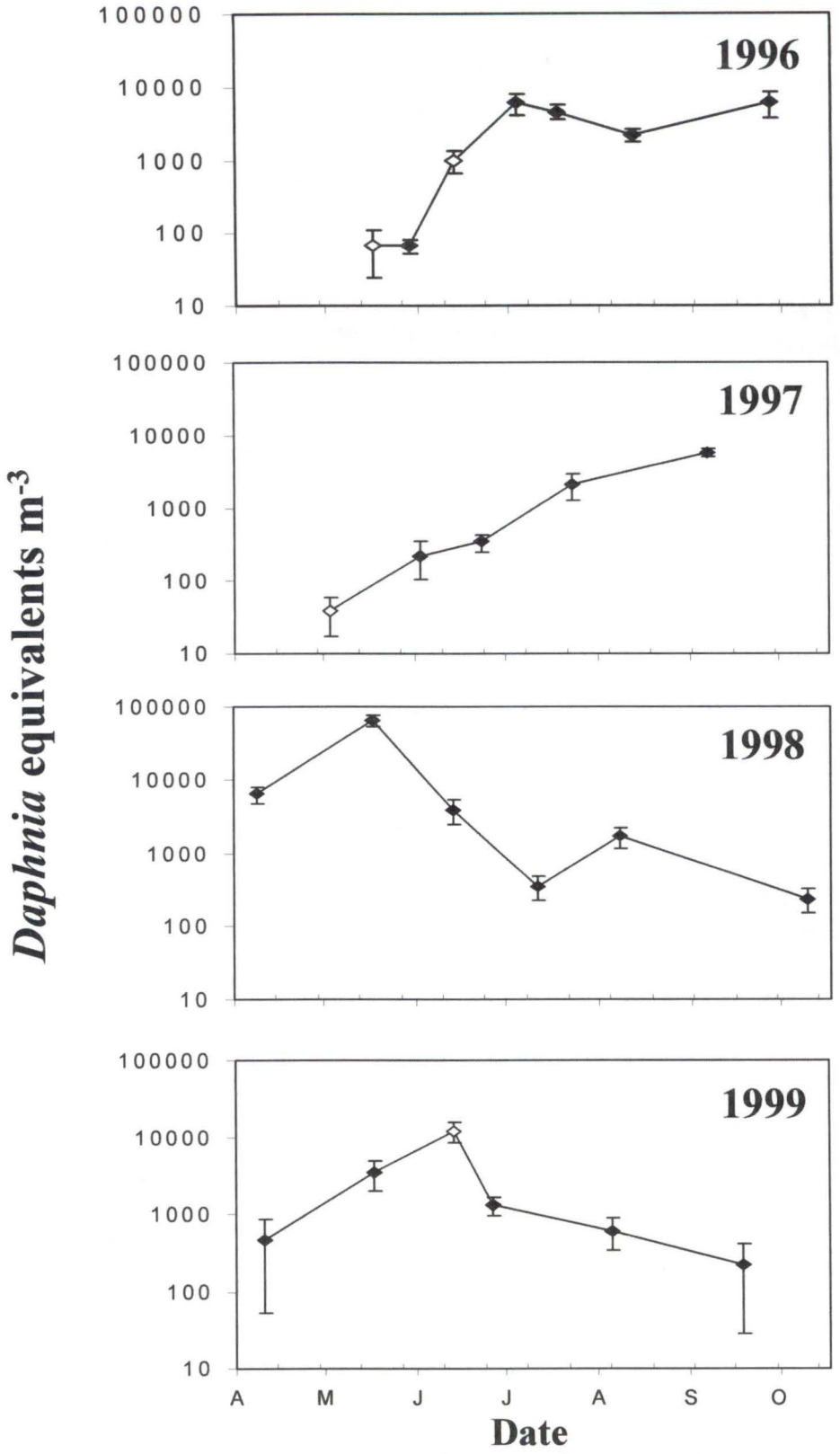

1996
1997
1998
1999
100000
10000
1000
100
10
A
M
J
J
A
S
O
Date
Daphnia equivalents m-3

Figure 9.

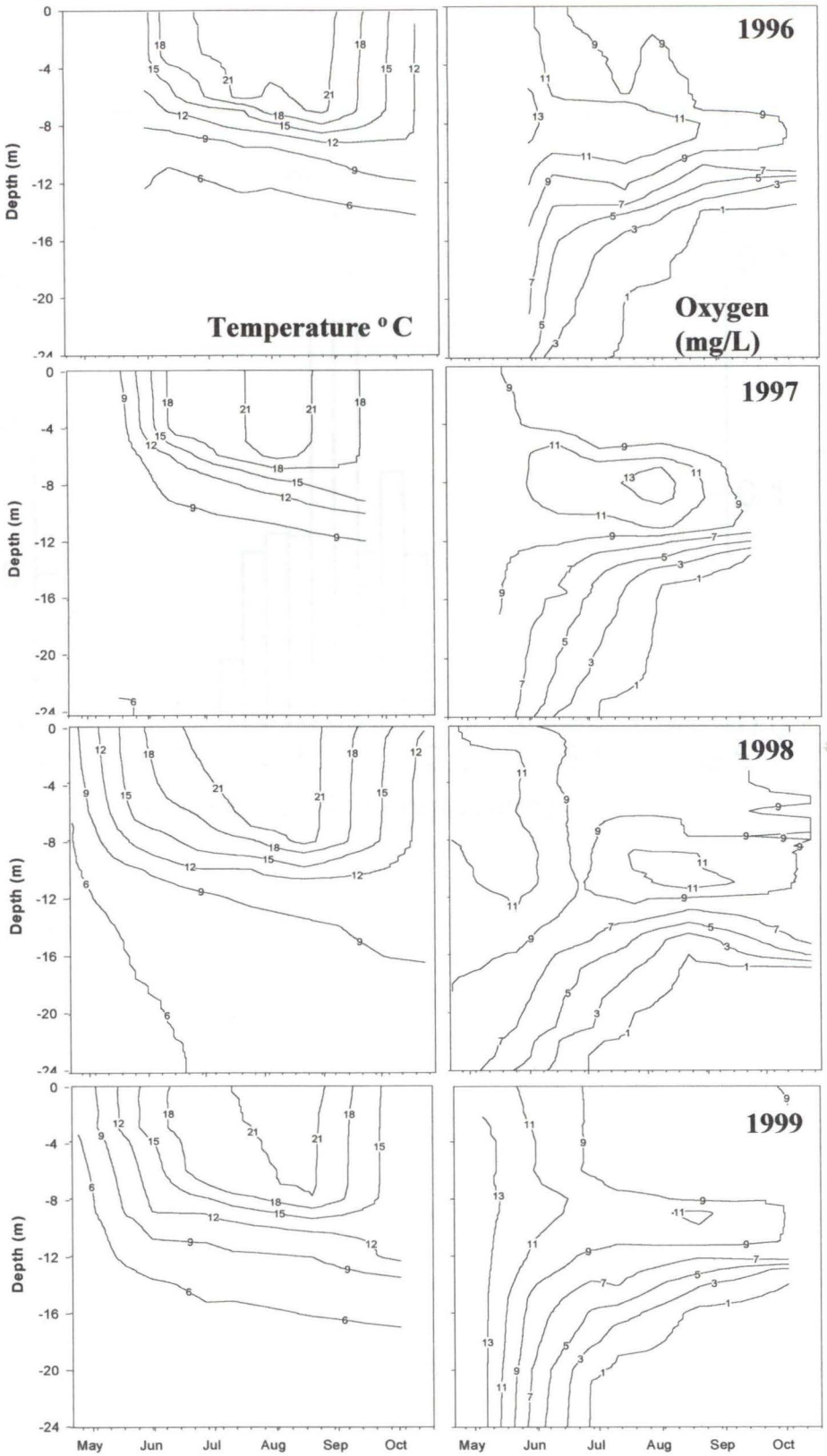

1996
1997
1998
1999
Temperature °C
Oxygen (mg/L)
Depth (m)
0
-4
-8
-12
-16
-20
-24
May
Jun
Jul
Aug
Sep
Oct

Figure 10.

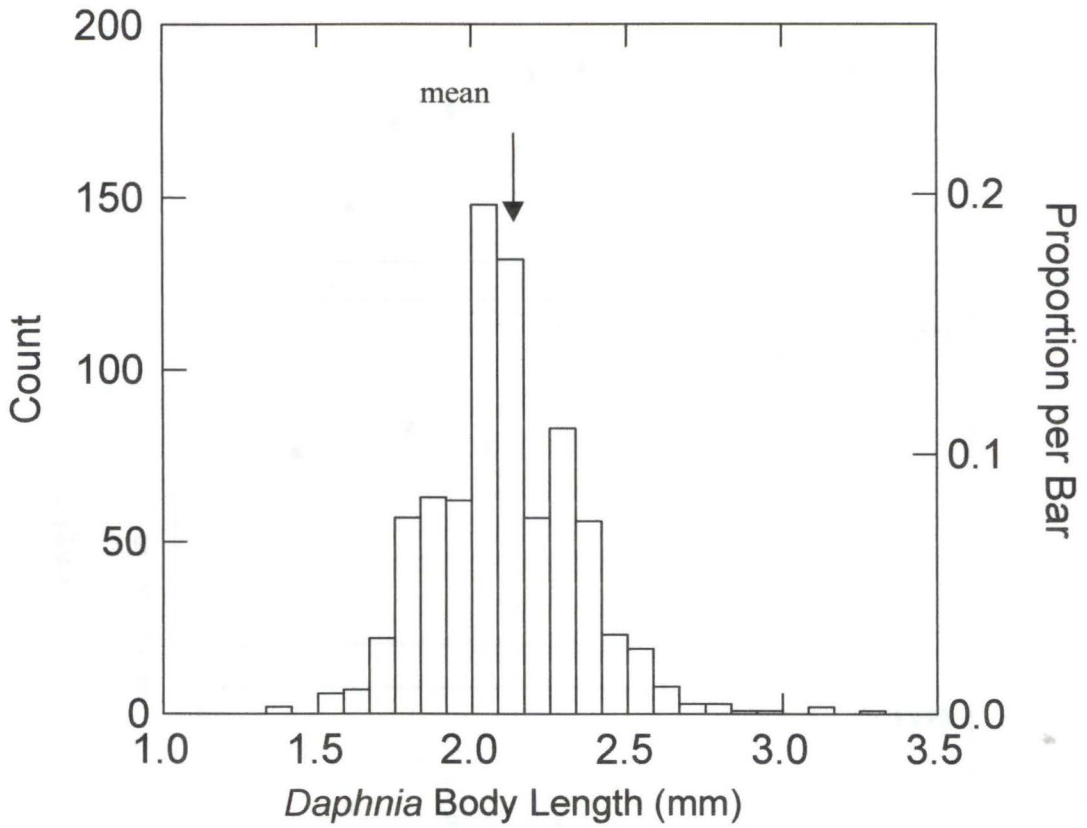

200
150
100
50
0
Count
mean
0.2
0.1
0.0
Proportion per Bar
1.0
1.5
2.0
2.5
3.0
3.5
Daphnia Body Length (mm)

Figure 11.

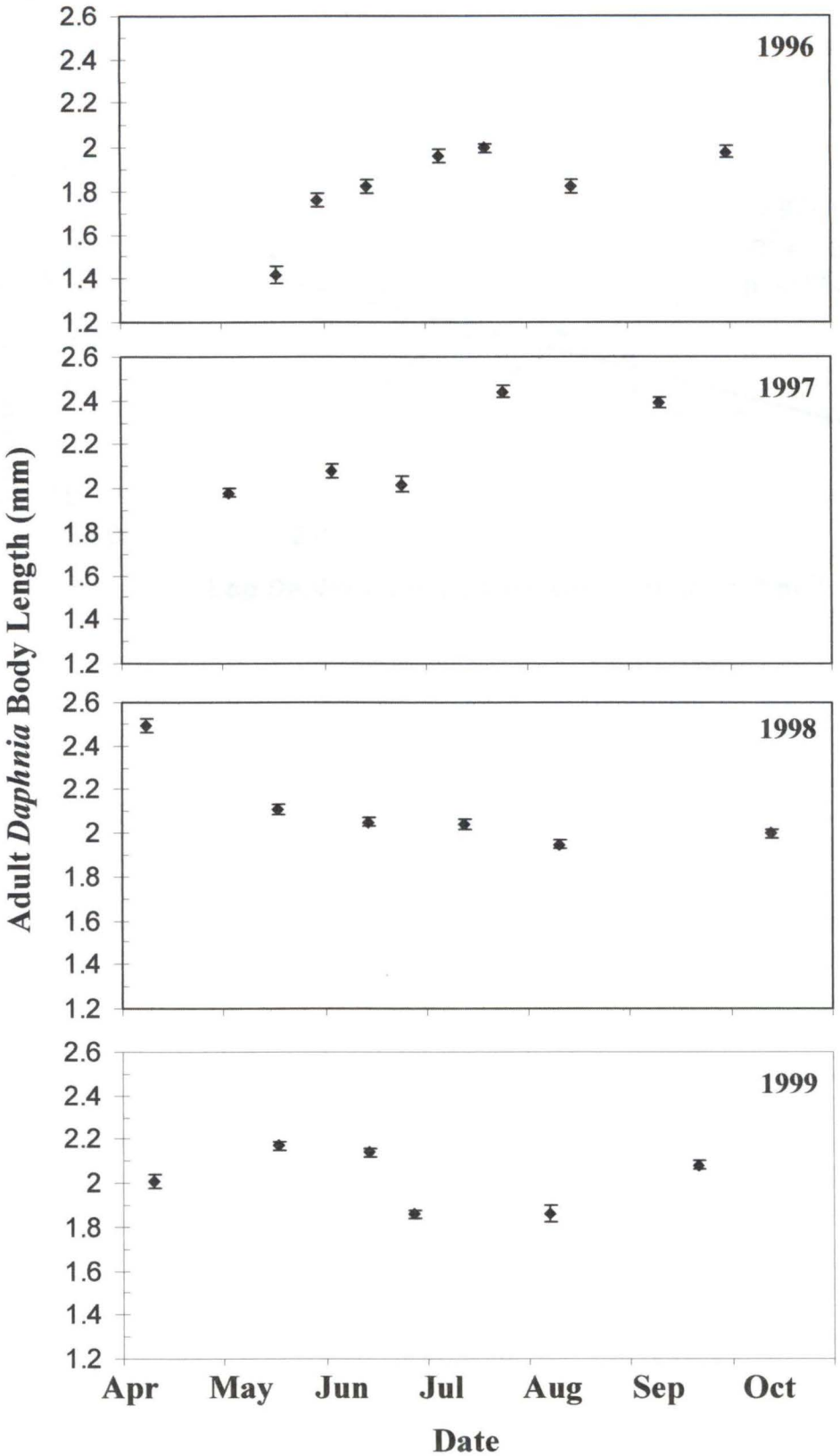

1996
1997
1998
1999
Adult *Daphnia* Body Length (mm)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
Apr
May
Jun
Jul
Aug
Sep
Oct
Date

Figure 12.

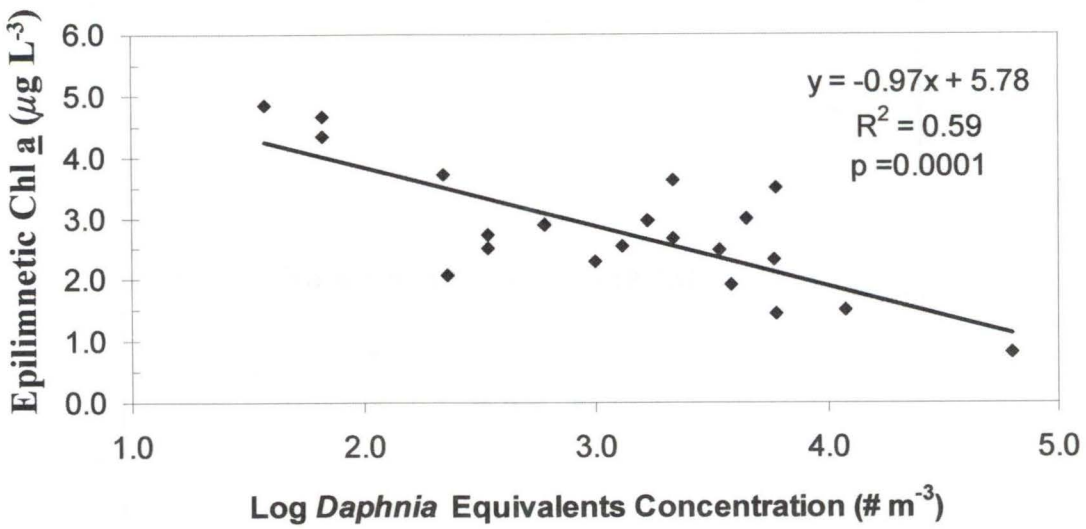

Epilimnetic Chl a (μg L-3)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1.0
2.0
3.0
4.0
5.0
y = -0.97x + 5.78
R2 = 0.59
p =0.0001
Log Daphnia Equivalents Concentration (# m-3)

Figure 13.

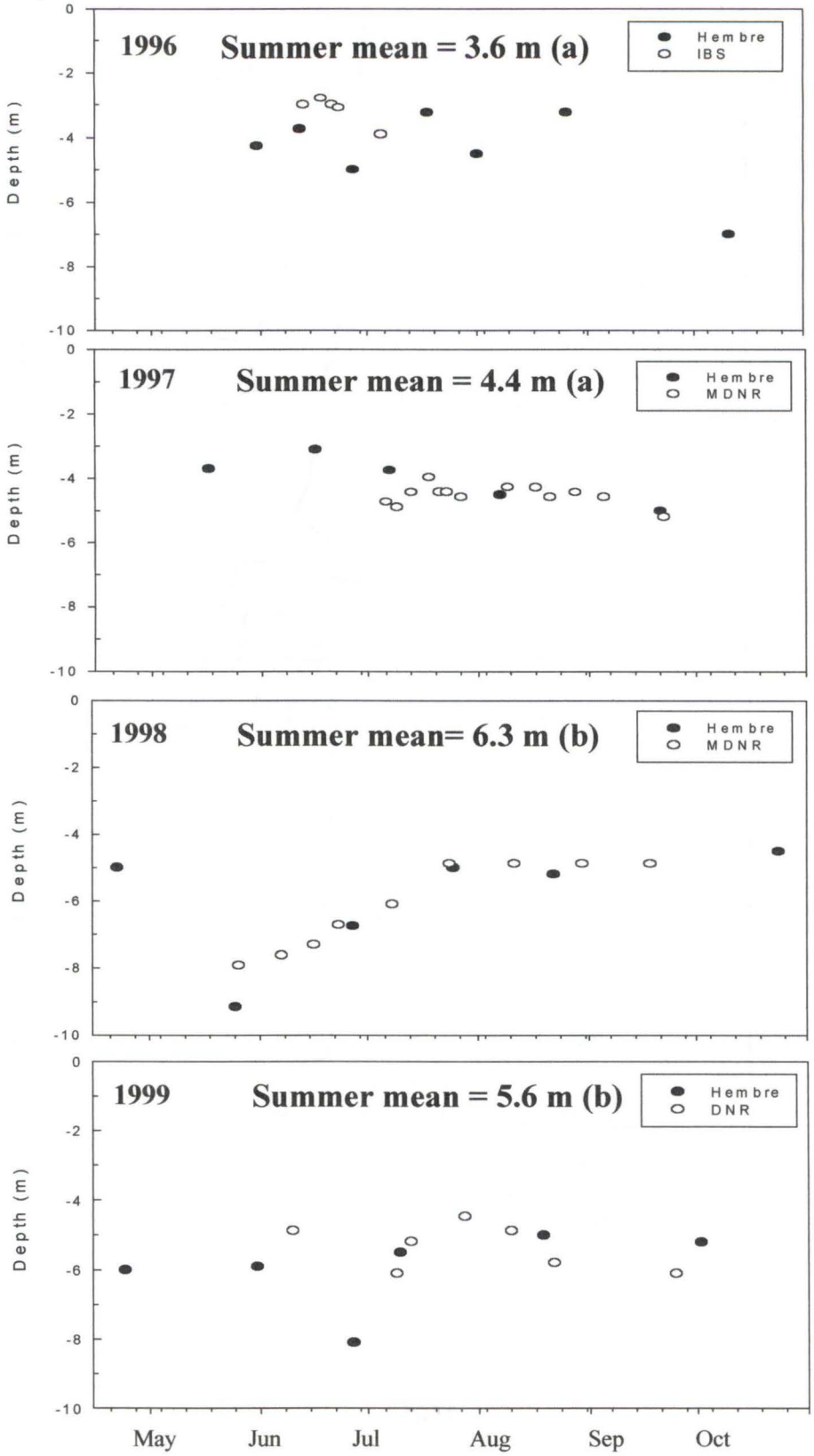

1996 Summer mean = 3.6 m (a)
Hembre
IBS
1997 Summer mean = 4.4 m (a)
Hembre
MDNR
1998 Summer mean= 6.3 m (b)
Hembre
MDNR
1999 Summer mean = 5.6 m (b)
Hembre
DNR
Depth (m)
0
-2
-4
-6
-8
-10
May
Jun
Jul
Aug
Sep
Oct

Figure 14.

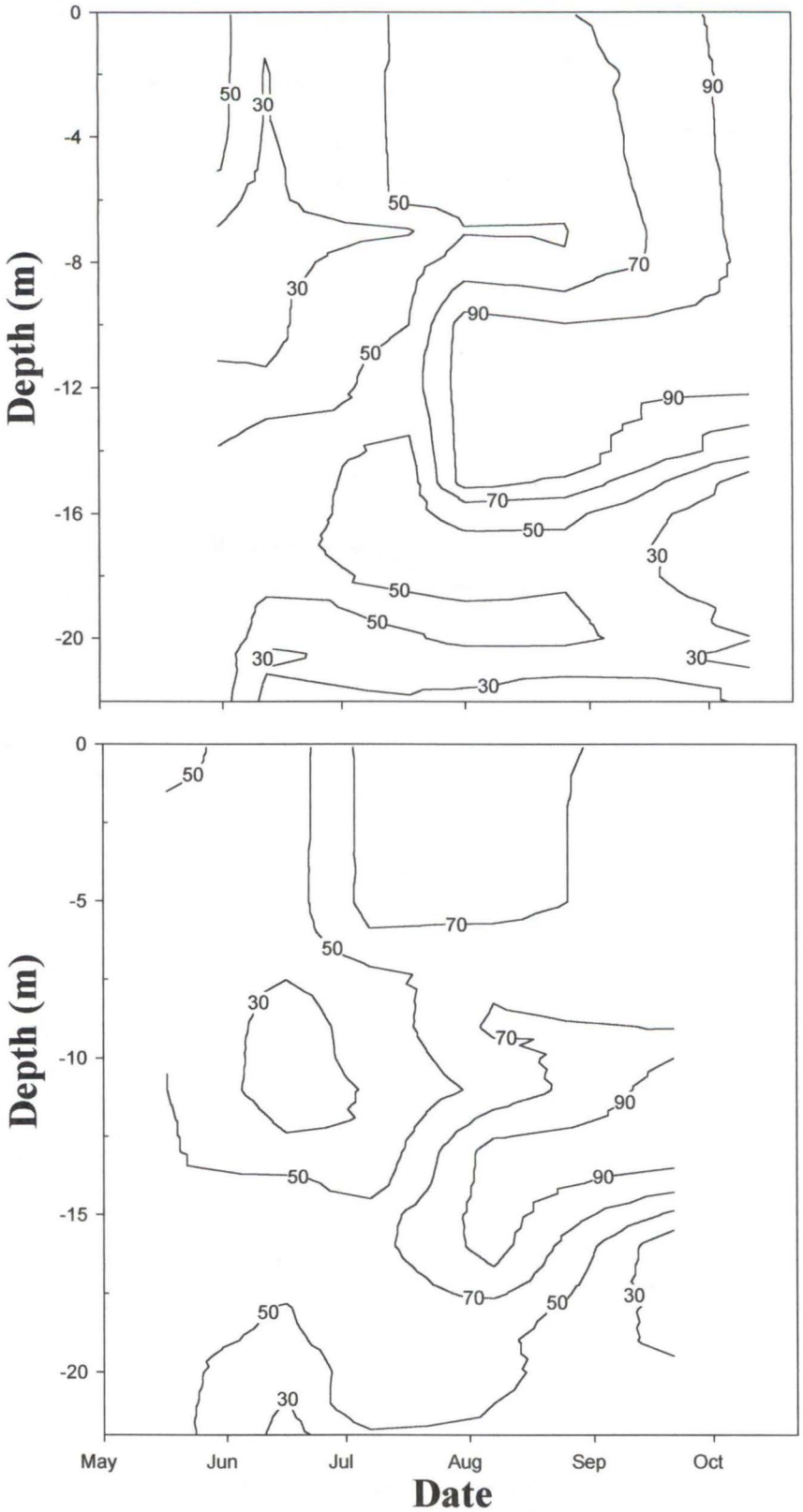
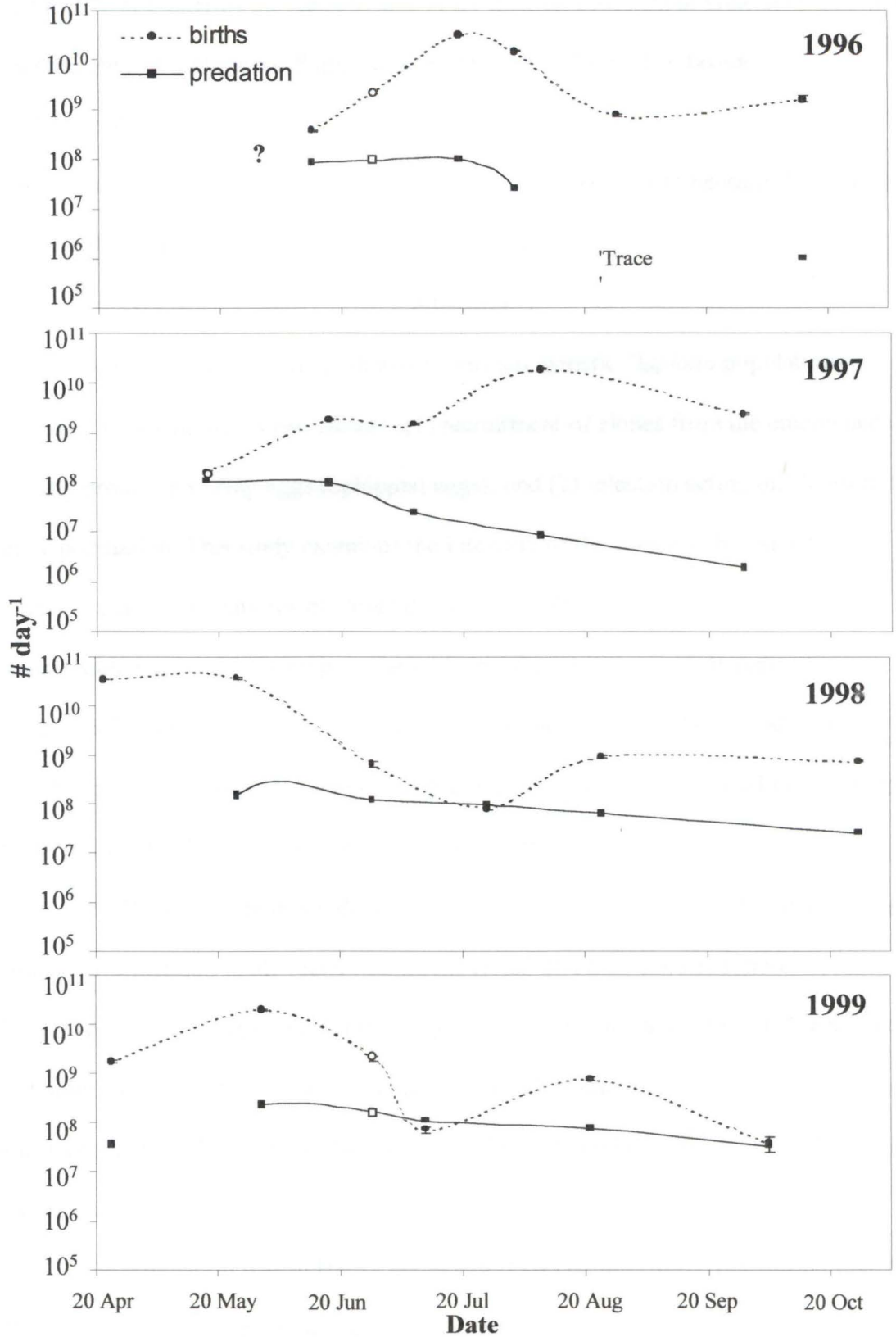

Depth (m)
0
-4
-8
-12
-16
-20
50
30
90
70
Depth (m)
0
-5
-10
-15
-20
May
Jun
Jul
Aug
Sep
Oct
Date

Figure 15.


births
predation
1996
1997
1998
1999
?
'Trace
day-1
20 Apr
20 May
20 Jun
20 Jul
20 Aug
20 Sep
20 Oct
Date

CHAPTER 3: Controls on Annual and Inter-annual Patterns of Genetic Structure and Diversity of a *Daphnia* Population Subjected to Trout Predation

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Abstract

The clonal diversity of facultatively parthenogenetic *Daphnia* populations depends fundamentally on two factors: (1) recruitment of clones from the emergence of sexually produced resting eggs (ephippial eggs), and (2) selection acting on clones in the active population. This study examines the interplay of these factors by exploring the genetic structure and patterns of clonal diversity of a *Daphnia pulicaria* population exposed either to weak or strong predation by rainbow trout over four years. Trout were stocked in the autumn of the first two years and in the spring for the second two years. The change from autumn stocking (AS) to spring stocking (SS) increased predation by trout on *Daphnia* during spring, summer, and autumn.

The *Daphnia* population differentiated genetically with respect to depth during summer stratification in all years. Maximum clonal diversity occurred in mid-summer of the AS years when environmental heterogeneity also was highest. Diversity decreased in mid-summer of the SS years. Genetic analyses of *Daphnia* in trout stomachs suggest that selective predation by trout on shallow water clones caused the mid-summer diversity decrease in SS years.

The population was in Hardy Weinberg (HW) equilibrium twice during this study. The active *Daphnia* population was at low density both times, suggesting that ephippial

recruitment is detectable only when the population is small. Two periods of clonal selection (CS) occurred when genotypes diverged from HW equilibrium. The switch in predation intensity due to the new stocking regime occurred during the second CS era. During the autumn stocking year of the second CS era, when trout were relatively scarce, the dominant clone had an affinity for shallower water. After the switch to spring stocking, when trout were more abundant, a deep-water clone became most abundant.

Results imply that clonal habitat partitioning and the strength of selection in microhabitats control annual patterns of clonal diversity, while periodic ephippial recruitment events that infuse genetic diversity into the population, and eras of clonal selection that ultimately reduce clonal diversity drive inter-annual patterns of diversity.

Introduction

Daphnia reproduction is primarily or exclusively parthenogenetic. Some populations are obligately parthenogenetic and reproduction is strictly asexual. Other populations are cyclical parthenogens, and clonal reproduction is periodically interrupted by sexual reproduction.

Cyclical parthenogens in temporary habitats (e.g., ephemeral ponds) have a repeatable pattern of reproduction. Populations establish as individuals emerge from diapausing sexual embryos contained in structures called ephippia. Individuals then reproduce parthenogenetically for a number of generations until the habitat begins to deteriorate. Environmental cues associated with the deterioration of the environment (Banta 1939; Stross and Hill 1965, 1968; Hobaek and Larsson 1990), or their interaction with intrinsic genetic factors (Yampolsky 1992; Deng 1996) trigger the production of males and of haploid eggs by females. Males fertilize the haploid eggs and the zygote undergoes several cell divisions before entering diapause in a structure called an ephippium. Females release ephippia when they molt, and the ephippia protect the embryos from desiccation and freezing until the habitat is renewed. For these populations, genotype frequencies usually agree with Hardy-Weinberg expectations (e.g., Hebert 1974a; Young 1979) though they may diverge if the period of parthenogenetic reproduction and clonal selection is sufficiently long.

In permanent habitats where *Daphnia* populations may persist year round (e.g., lakes), the pattern of alternation between parthenogenesis and sexual reproduction is less predictable (Hebert 1974b, 1987; Lynch 1983). The timing of sexual reproduction is less predictable, and the significance of ephippial recruitment is less clear. Because the

pattern of reproduction is not as seasonal as it is for populations in temporary habitats (Lynch 1984), these populations are more accurately described as "facultative" rather than "cyclical" parthenogens.

As the duration of clonal reproduction increases in a facultatively parthenogenetic population, the influence of sexual reproduction diminishes and genotypic proportions begin to deviate from Hardy-Weinberg equilibrium (e.g., Lynch 1983; Jacobs 1990; King et al. 1995). Under these circumstances, the population effectively operates like an obligately parthenogenetic population in which clonal selection principally controls genetic composition (Hebert and Crease 1980; Lynch 1983).

Maintenance of Clonal Diversity in the Absence of Sex

In a population that reproduces only parthenogenetically, one may expect clonal selection to eliminate all but one or a few of the best-adapted clones. Contrary to this expectation, however, studies of exclusively parthenogenetic populations from a variety of taxa commonly find many coexisting clones (e.g., Hebert 1978 in *Daphnia*; Janike et al. 1980 in *Octalasion* earthworms; Fox et al. 1996 in *Potamopyrus* snails; Weeks and Hoffman 1998 in *Penthaleus* mites).

Two hypotheses with similar predictions have been put forth to explain this evolutionary puzzle. One is that the relative fitnesses of the coexisting clones are unstable, and that environmental changes in space and time shift genotypic fitnesses before competitive exclusion occurs (Hebert and Crease 1980). The other, the *frozen niche variation hypothesis* (Vrijenhoek 1979, 1984) holds that clones are rigidly adapted to particular ecological niches. The existence of multiple microhabitats therefore

promotes and maintains clonal diversity. Both hypotheses invoke environmental heterogeneity and varying selection regimes to explain coexisting clones in asexual populations.

The notion that environmental heterogeneity promotes clonal coexistence is supported by several studies that have documented spatial and temporal habitat partitioning by *Daphnia* clones (Weider 1984, 1985; Carvahlo and Crisp 1987a; Jacobs 1990; Muller and Seitz 1993; Geedy et al. 1996; Tessier and Leibold 1997). Mechanisms proposed to control habitat partitioning include differential tolerances to environmental factors such as temperature (Carvahlo and Crisp 1987b), oxygen (Ross et al. 1996), salinity (Weider and Hebert 1987), or different vertical migration strategies (Weider 1984; Stirling et al. 1990; Muller and Seitz 1993; King and Miracle 1995).

Mechanistic support for field observations of habitat partitioning has been provided by laboratory experiments with *Daphnia* clones. Studies have demonstrated differences among clones in (1) phototactic behavior (Dumont et al. 1985), (2) phototactic response in the presence and absence of predator kairomones (De Meester 1993a; De Meester and Cousyn 1997), and (3) the ability to produce hemoglobin and tolerate low oxygen concentrations (Weider and Lampert 1985).

Effect of Predation on Clonal Composition

An assemblage of asexual *Daphnia* clones is analogous to a zooplankton community comprised of different species. Predation and competition principally structure zooplankton communities (Brooks and Dodson 1965; Zaret 1980; Kerfoot and Sih 1987) and should therefore also structure the clonal composition of *Daphnia*

populations. The effect of predation by fish on zooplankton is well known. Most planktivorous fish are visual predators that selectively feed on conspicuous targets. The most obvious targets are usually the largest zooplankters, but other factors such as pigmentation, evasiveness, and depth selection can be important. When planktivorous fish are abundant, large-bodied zooplankton (e.g., large *Daphnia*) are removed preferentially and the zooplankton assemblage tends to be dominated by smaller-bodied forms (e.g., rotifers, small cladocerans, and copepods). Conversely, when planktivorous fish densities are low, larger-bodied species (e.g., large *Daphnia*) typically dominate the zooplankton assemblage (Brooks and Dodson 1965; Hall et al. 1976; Gliwicz 1990; Kreutzer and Lampert 1999).

While the effect of fish predation on zooplankton communities has been well studied, few studies have directly evaluated how predation affects the genetic composition of populations (Exceptions: Tessier et al. 1992; Pijankowska et al. 1993). Pijankowska et al. (1993) demonstrated that predators can change clone frequencies in mixed cultures of *D. magna* in the laboratory. The two clones used in this study originated in different habitats - one was fishless, and the other inhabited by fish. The clone isolated from a fishless pond was found to be more positively phototactic and significantly more susceptible to predation by fish in aquarium experiments than the clone isolated from the pond with fish. Tessier and Leibold (1992) documented body-size selection and an overall reduction in genetic variability in a *Daphnia galeata mendotae* population in a lake presumed to have abundant vertebrate predators. This study clearly shows significant genetic changes in the *Daphnia* population over the summer, but does not provide direct evidence that fish predation caused the changes they observed.

Study Site & Fisheries Management

This study examines the genetic composition of a *Daphnia pulicaria* population in a lake in northwestern Minnesota (Long Lake, Ch. 2, Fig.1) that is annually stocked with rainbow trout, a predator of *Daphnia*. The lake is classified as mesotrophic to oligotrophic (Moyle 1969), and is more transparent than most other lakes in the region. During summer stratification, light is sufficient to support substantial photosynthetic activity in the metalimnion where oxygen concentrations are highest. Oxygen decreases steadily in the hypolimnion and disappears in late summer. For more details on characteristics of the lake see Chapter 2. Long Lake's cold, well-oxygenated metalimnion provides suitable habitat for rainbow trout (*Oncorhynchus mykiss*), which has been stocked annually since 1961 by the Minnesota Department of Natural Resources (MDNR). Rainbow trout require streams with current-washed gravel to spawn, and since such streams are not available to the trout in Long Lake, natural reproduction does not affect trout abundance. Instead, trout abundance is determined by the number of fish that are stocked, natural mortality, and mortality due to fishing.

For the first two years of this study (1996-1997), trout were stocked the preceding fall as fingerlings. Because winter survival was low, trout were not added in the autumn of 1997. Instead, for the next two years, the trout were stocked in the spring, soon after ice-out. The same number of fish (14,500) was stocked each year (Ch. 2, Table 1).

Though the stocking rate was the same for all years, the change from autumn to spring stocking altered the predation regime experienced by the *Daphnia* population. In years after fall stocking (1996-1997), *Daphnia* was subjected to the most severe trout

predation during winter. After spring stocking, predation by trout on *Daphnia* was most intense during the spring and summer.

Expectations and Evaluation

The switch from fall to spring stocking of rainbow trout provided an opportunity to examine how intensified predation during the open-water season would affect the genetic structure of the *Daphnia pulicaria* population in Long Lake. The following are our expectations and methods for evaluation.

I. Clones specialize on particular microhabitats (Vrijenhoek 1979, 1984), and the increased environmental heterogeneity associated with summer stratification should promote habitat partitioning among clones.

Evaluation: We evaluated this expectation by using χ^2 tests of heterogeneity to compare the clonal composition of samples collected from different depths to determine if and when habitat partitioning occurred. We also compared the frequencies of individual clones from the shallow- and deep-water samples to determine whether particular clones had consistent habitat affinities.

II. Rainbow trout are planktivorous, size-selective, predators (Galbraith 1967; Kitchell and Kitchell 1980; Geist et al. 1993; Wang et al. 1996; Hirsch and Negus 2000), require water colder than 21° C, and dissolved oxygen concentrations greater than 5 mg/L (Wang et al. 1996). Together, their feeding mode and the environmental constraints on their distribution should result in selective predation on large zooplankton (i.e., *D. pulicaria*) at

depths within the bounds of their habitat. During the summer stratification period, this should largely restrict trout predation to the metalimnion. Therefore, metalimnetic *Daphnia* clones should have higher predation mortality than those inhabiting the darker, less oxygenated waters of the hypolimnion. Deep-water clones, therefore, should have a selective advantage and increase in frequency when predation is high.

Evaluation: We surveyed the spatial distribution of the trout with sonar, and analyzed the stomach contents of trout to determine their diet. To determine whether the trout were selectively feeding in the metalimnion, the genetic composition of the *Daphnia* in trout stomachs was compared with that of *Daphnia* from different depths in the water column using χ^2 tests of heterogeneity. We also monitored clone frequencies in the water column samples to determine whether clones with particular habitat affinities increased or decreased under the different predation regimes.

III. Environmental heterogeneity and varying selection in space and time are expected to promote and maintain clonal diversity. Therefore, as the environment becomes more heterogeneous (e.g., during summer stratification) clonal diversity should increase. The converse should also be true. That is, reduced environmental heterogeneity or consistent, strong selection should cause a decline in clonal diversity. We expected that the heavy predation by rainbow trout during years of spring stocking would impose strong selection on metalimnetic clones and thereby decrease clonal diversity.

Evaluation: We quantified clonal diversity using Simpson's Diversity index (1/C), where $C = \Sigma(p_i^2)$, p_i is the frequency of the i^{th} clone in the sample, and the summation of squares is over all clones (Weider 1992; Fox et al. 1996). This index is sensitive to clonal richness (i.e., the number of clones identified in the sample) and the evenness of their proportional abundance. The value of the index ranges from a minimum of 1 if all individuals in the sample are the same clone type, to a maximum that is equal to the number of clones possible in the sample. The maximum value occurs if all clones are present in the sample, and are at the same frequency. We compared the patterns of clonal diversity after fall stocking (1996-1997) with those after spring stocking of trout (1998-1999) to assess whether intensified predation and selection against metalimnetic clones decreased clonal diversity.

Methods

Use of Sonar System

The spatial distribution and abundance of zooplankton and fish were mapped with a sonar system that consists of a Lowrance X-16 high-frequency (192 kHz) single-beam echosounder and a Loran-C navigation receiver connected to an IBM personal computer. For details about the configuration of the system and its operation see Chapter 1. The volume scattering strengths are displayed instantaneously on the computer monitor as echograms and the echograms were used to locate aggregations of zooplankton and to select depth increments for net. In addition to providing instantaneous information for plankton net sampling, the sonar data were saved on the computer's hard disk and analyzed to estimate the abundance of *Daphnia* in the lake (Chapter 1).

Sonar Estimation of Daphnia and Trout Abundance

To evaluate zooplankton distribution and abundance, a narrow beam (4° half angle) transducer was directed vertically from the bow of the boat, and acoustic data were collected while traveling at about 5 km h^{-1} along a transect of the lake's long axis from the southeast to the northwest end of the lake (Ch. 2, Fig. 2).

To obtain information about the abundance of rainbow trout, we performed a series of "zig-zag" transects (Ch. 2, Fig. 2) using a wide-beam (10° half angle) transducer. The wider beam angle of this transducer provides a clearer image of the characteristic arc-shaped fish echo than does the narrow-beam transducer. To estimate trout abundance, fish echoes were counted from echograms at depths within the bounds of the rainbow trout habitat (i.e., depths with temperature < 21°C, and oxygen > 5 mg/L). On dates when zig-zag transects were not done, we estimated trout abundance by counting fish echoes

from the long-axis transects obtained using the narrow beam transducer (Ch. 2, Fig. 4). For the narrow beam transects, we estimated trout abundance by filtering out the weaker echoes (< -70 dB) and counting fish traces in the depth interval containing viable trout habitat. See Chapter 2 for details about how the different sonar methods were standardized to assess trout abundance.

Environmental and Zooplankton Sampling

We sampled Long Lake approximately monthly during the open-water seasons of 1996-1999, and once in 2000 (17 May). Sampling was conducted during the day (between 11 a.m. and 5 p.m.) at a deep (24 m) location in the middle of the lake (Ch. 2, Fig. 2). Profiles of temperature and oxygen concentration were collected on all dates using a YSI Model 58 meter at 1 m intervals, and we sampled zooplankton from discrete depth increments with vertical tows of a closing Wisconsin-style plankton net (27 cm diameter, 130 μm mesh size).

The bucket of the net was fitted with a 130 μm filter to collect samples for enumeration that were used to calibrate the sonar information (Chapter 1). An 800 μm filter, that allowed smaller bodied zooplankton to pass, was used to collect *D. pulicaria* for genetic analyses. On most dates, we obtained three samples for genetic analyses: (1) a vertical tow of the entire water column, (2) a sample from deep water (usually the hypolimnion), and (3) a sample from shallower water (usually in the metalimnion) (Table 1).

Samples of *D. pulicaria* for genetic analysis were refrigerated and analyzed within 48 h, or frozen at -70 $^{\circ}$C in 96-well plates for future analysis. Samples collected

for enumeration were preserved in cold sugar-formalin (Prepas 1978), and counted using a stereomicroscope.

Trout Sampling

On eight dates (Table 1), we obtained rainbow trout or trout stomachs from anglers for gut content analyses. The stomachs were dissected in the laboratory and the contents were gently rinsed over a sieve (230 μm mesh) with distilled water. Individual *Daphnia* were then removed from the rinsed samples for immediate allozyme analysis, or frozen for future analysis.

Electrophoretic Analyses

Daphnia individuals from each sample were analyzed for pgi (phosphoglucose isomerase, EC 5.3.1.9) and pgm (phosphoglucomutase, EC 2.7.5.1) using cellulose acetate electrophoresis and following the methods of Hebert and Beaton (1989). We identified 4 alleles for pgi (Slow (S), Medium (M), Medium-Fast (MF), and Fast (F)) and two alleles for pgm (Fast (F) and Slow (S)). For this level of allelic variation, 30 different 2-locus electromorphs are possible. Two-locus electromorphs are hereafter referred to as clones, with the understanding that they may represent multiple clones with common genotypes at these markers. Previous research on the population of *Daphnia* in Long Lake (Ross et al. 1996) confirmed its identity as *D. pulicaria* by analyzing a sample for Ldh (EC 1.1.1.27), which is a diagnostic allozyme locus for distinguishing between *D. pulicaria* and *D. pulex* (Cerny and Hebert 1993).

Results

Daphnia Population Dynamics and Spatial Distribution

Spring and fall stocking of trout induced very different *Daphnia* dynamics (Fig. 1). *Daphnia* were sparse during the spring (< 100 m^{-3} in May and early June) after fall stocking, increased during the summer, and were most abundant (~ 6000 m^{-3}) in the fall. When trout were not added to the lake in the fall, spring concentrations of *Daphnia* were substantially larger than in the previous two years. Trout were stocked within a week after the April sampling dates in 1998 and 1999, but despite predation by the newly stocked trout, the *Daphnia* populations grew. In both years *Daphnia* were most abundant in late spring (25 May, 1998) or early summer (27 June, 1999) at very high densities (>10000 m^{-3}). They decreased during the summer, and were least abundant in October (<250 m^{-3}).

With few exceptions (11 October 1996, 21 September 1997, and 22 April 1998) the population was distributed below the epilimnion (Ch. 2, Fig. 7) during the day and above depths where oxygen concentrations were less than 3 mg/L (see Fig. 2).

Trout Abundance

Trout were most abundant during spring and summer after spring stocking (Fig. 3). In 1996 and 1997, trout abundance was moderate in early summer, but decreased to very low levels in the late summer and fall (August - October). In 1998 and 1999 trout abundance declined from peak levels in May to minimum levels in October, but maintained substantially higher levels through the summer than in 1996-1997. Trout abundance was very low (< 160) on the three dates (11 October 1996, 21 September 1997, and 22 April 1998) when we observed *Daphnia* in surface waters during the day.

Daphnia Genetics

All 30 of the clone types possible, given the allelic variation at the pgi and pgm loci, were sampled over the course of this study from 1996-2000 (Table 2). Data from 1995 from a previous study of the same population (Hembre 1996) identified only 10 of the 30 clones, with the vast majority (~ 96%) of the individuals belonging to just three clones. In 1996, 23 clones were sampled and clonal proportions were much more even than in 1995. The number of different clones sampled remained high ($\geq$ 24) in 1997, 1998, and 1999. However, by 1999, two clones accounted for ~72% of the individuals sampled. Thirteen clones were identified on 17 May 2000, and two of them constituted 93% of the population. The frequencies of common clones at various depths are shown in Table 3.

Genetic Structure of the Population

A similar pattern of genetic differentiation emerged each year. Usually, clonal proportions did not vary with respect to depth during the spring and early summer, but as environmental stratification became more pronounced (Fig. 2) there was significant differentiation of the population (Table 4). In 1997, *Daphnia* were only found in deep water early in the year (Chapter 2, Fig. 7b.), so it was not possible to investigate habitat partitioning until August.

To investigate whether particular clones exhibited preferences for particular depths, the frequencies of individual clones in shallow and deep water were compared (Table 5). In 1996, clones 10 and 19 were proportionally more abundant in deep water during late summer, while clone 4 was significantly more common in shallow water. Clone 14 did not display a consistent affinity for either habitat.

Two clones displayed consistent habitat affinities during 1997-1999. Clone 19, identified as a deep-water clone in 1996, was typically more abundant in shallow water, and clone 17 regularly occurred at higher frequencies in the deep-water samples. The other two common clones in 1997-1999 (clones 7 and 14) did not show consistent habitat selection.

Selective Predation by Trout

Trout were selective predators of *Daphnia pulicaria* at the population and subpopulation level. Trout consumed *D. pulicaria* in much higher quantities than other zooplankters (Ch. 2, Table 3). Genetic analyses of *Daphnia* from trout stomachs also revealed a selective preference by trout for shallow water clones. The clonal composition of *Daphnia* in trout stomachs always was significantly different ($p < 0.05$) from that of *Daphnia* in deep-water samples, but never differed significantly from that of *Daphnia* in shallow water (Table 6). These results imply that deep water provided a refuge from trout predation for *Daphnia*.

Annual Patterns of Clonal Diversity

The pattern of clonal diversity (Fig. 4) after fall stocking differed from that after spring stocking. Clonal diversity in 1996 and 1997 mirrored habitat diversity. Diversity increased with the onset of stratification to a maximum in July, as oxygen concentrations decreased (< 5 mg/L) in the hypolimnion (Fig. 2). Diversity subsequently decreased as the habitat available to *Daphnia* decreased in late summer due to hypolimnetic anoxia (< 1 mg/L) and the deepening of the epilimnion.

Unlike the years after fall stocking, diversity decreased as the water column differentiated in late June to early July after spring stocking (1998-1999). Abundant trout

(Fig. 3) and selective predation on metalimnetic clones (Table 6) could account for the observed mid-summer decreases in clonal diversity. Since the diversity index is sensitive to the evenness of clones as well as richness, the selective removal of metalimnetic clones would have decreased their relative abundance and made the distribution of clones less even.

Evidence for Sexual Reproduction

To evaluate whether the emergence of sexually produced individuals from resting eggs affected the genetic composition of the *Daphnia* population, genotype frequencies at the two allozyme loci were compared with Hardy-Weinberg (HW) equilibrium expectations. For all but four dates (12 and 27 June 1996 and 16 June and 7 July 1997) genotype frequencies at both loci differed significantly from those expected under HW equilibrium (Table 7).

The agreement with HW expectations observed in June of 1996 and the striking increase in clonal richness in 1996 compared to 1995 (Table 2) strongly suggest that the population had recently established from ephippia. On subsequent dates in 1996, genotype frequencies differed significantly from H-W expectations implying that the population was undergoing clonal selection.

On the first sampling date in 1997 (17 May), pgi and pgm genotype frequencies deviated from H-W expectations. However, data from the next two sampling dates in 1997 (16 June and 7 July) indicate that the genetic composition of the water column population was again significantly affected by immigration from the egg bank. On 16 June 1997 one of the two loci (pgm) did not show deviation from H-W equilibrium, and on 7 July 1997 neither locus deviated from H-W expectations. The incomplete agreement

with H-W expectations in June was likely due to an amalgamation of clones surviving from May, and new clones emerging from sexual ephippia. Hardy-Weinberg agreement at both loci suggests that ex-ephippial immigrants comprised the majority of the population by July of 1997. On all dates after 7 July 1997 genotype frequencies for both allozyme loci differed significantly from H-W expectations (Table 7) implying that clonal selection primarily controlled the genetic composition of the population.

Clonal Selection Eras and Emergence Events

The *D. pulicaria* population in Long Lake alternated between eras of clonal selection and ephippial emergence events from 1996-2000 (Fig. 5). The first clonal selection era (CS1) followed an ephippial emergence event in June of 1996 (HW1), and lasted until May of 1997. Genotype frequencies diverged from HW equilibrium (Table 7), during this era and clones segregated with respect to depth (Tables 4 & 5). Clone 4 was consistently more abundant in shallower water, while clones 10 and 19 displayed affinities for deep water. The second ephippial emergence event (HW2), detected in June-July of 1997, was followed by a second clonal selection era (CS2) that was in progress at the end of the study (17 May 2000). The intensity of predation by trout on *Daphnia* during summer stratification changed dramatically during CS2 as a result of the switch from autumn to spring stocking of trout. From 7 August 1997 to 22 April 1998 trout abundance was very low (Figure 2). During this period (CS2a) the most abundant clone (clone 19) was a shallow-water clone. After the initiation of spring stocking on 23 April 1998, trout abundance was markedly higher than it was from August 1997 to April 1998 (Fig. 2). From May of 1998 to May of 2000 (CS2b), clone 19 decreased to an undetectable level, and a deep-water (clone 17) increased to become the most abundant

clone in the population. The shift from the dominance of a shallow-water clone to a deep-water clone supports our expectation that selection would favor deep-water clones under heavy predation by rainbow trout.

The habitat affinity of clone 19 switched from a deep-water affinity during CS1 to a shallow-water affinity during CS2. Considering that a new cohort of clones emerged from ephippia in June-July 1997, it is likely that the clone 19 from the CS2 era was a different clonal lineage from the clone 19 of the CS1 era. Although other studies have shown physiological relevance of allozyme loci (e.g., Watt et al. 1985- *Colias* butterflies) our data suggest that the allozyme genotypes of the clones are neutral genetic markers and are not themselves relevant to the physiology of the individuals.

Inter-annual Patterns in Clonal Diversity

A plot of clonal richness on sampling dates from 20 June 1995 to 17 May 2000 shows the long-term pattern of clonal variation of the *D. pulicaria* population in Long Lake (Fig. 6). Clonal richness was examined instead of clonal diversity (1/C), because the entire water column was not sampled in 1995 (Hembre 1996) and the diversity index could not be calculated in the same way as in other years. Instead, clonal richness of samples pooled from different depths in 1995 was compared to the richness of clones in the water column tows from 1996-2000. While patterns of clonal diversity within a year appear to be driven by environmental heterogeneity, habitat partitioning, and the strength of selection in different microhabitats (Fig. 4), the arch-shaped pattern revealed by this plot (Fig. 6) suggests that interactions of clonal selection and clonal immigrations from the egg bank control clonal diversity in the longer term. Clonal richness was low in 1995 (3 -7 clones sampled on a given date) probably due to a long period of clonal selection.

Ephippial emergence injected new clones to the population in 1996 and 1997 and increased richness (8-15 clones identified). During the period of clonal selection that followed (CS2 - Fig. 5), clonal richness declined. A regression of clonal diversity versus time during CS2 (Fig. 7) also shows a significant decrease ($p = 0.0046$) over this time period.

Discussion

The genetic structure and diversity of the *D. pulicaria* population in Long Lake from 1996-2000 was affected both by clonal selection and ephippial recruitment. Unlike a cyclically parthenogenetic population in a temporary habitat, however, the alternation between clonal selection and ephippial recruitment was not annual. Instead, recruitment from the egg bank was significant only twice (Table 7), with clonal selection otherwise operating as the dominant evolutionary force.

During the periods of clonal selection, environmental heterogeneity and predation by trout combined to control the genetic composition of the population. In all years, environmental heterogeneity during summer stratification promoted habitat partitioning by clones within the population (Table 4). Trout predation acting on this environmental backdrop affected both the clonal diversity and clonal composition of the population. During years after autumn stocking (1996-1997), predation was relatively low during the summer (Fig. 3), and clonal diversity mirrored environmental diversity (Figs. 2 & 4). In the transition between autumn and spring stocking (August 1997 - April 1998), when trout were least abundant, the frequency of a shallow-water clone (C19) increased (Fig. 5). After spring stocking, when trout predation was strong during the summer (Fig. 3), *Daphnia* clones were selectively removed from metalimnetic water inhabited by trout (Table 6). The frequency of the shallow-water clone (C19) decreased precipitously after spring stocking, and it was replaced by a deep-water clone (C17) as the most abundant clone in the population (Fig. 5). Selective predation on metalimnetic clones also likely caused clonal diversity to decrease in early summer of the spring stocking years (Fig. 4).

A demographic analysis of the *Daphnia* population (Chapter 2) supports the hypothesis that trout predation was responsible for the mid-summer decreases in clonal diversity in 1998-1999 (Ch. 2, Fig. 15). This analysis showed that population losses caused by trout predation exceeded *Daphnia* production during times in 1998 and 1999 when clonal diversity was at its lowest.

The observations of habitat partitioning (Table 4), microhabitat specialization of clones (Table 5), and the correlation between clonal diversity and environmental diversity (Figs. 2 & 4) support the predictions of the frozen niche variation hypothesis (FNVH) (Vrijenhoek 1979, 1984). These results imply that clones show niche specialization, and that increased habitat diversity promotes increased clonal coexistence. That is not to say, however, that clones are "rigidly" adapted to specific ecological conditions. Important adaptations such as the phototactic response of *Daphnia* to fish kairomones (De Meester 1993a; De Meester and Cousyn 1997) and the production of hemoglobin (Weider and Lampert 1985) are known to be inducible and considerably plastic about their genetic value, and this is undoubtedly also true for *Daphnia* in Long Lake.

While the patterns of the clonal diversity observed in 1996 and 1997 (Fig. 4) are consistent with the FNVH, an alternative explanation is that injection of new clones from the egg bank caused mid-summer maxima in clonal diversity. In the longer-term (i.e., among years), the ephippial recruitment that occurred in 1996 and 1997 did result in increased clonal diversity (Fig. 6). However, patterns of diversity within each of these years apparently depended on clonal selection in the water column. In both years, clonal diversity reached its seasonal maximum after the population diverged from Hardy-Weinberg equilibrium, not while it was in equilibrium (Table 7).

These results are also consistent with the idea of Hebert and Crease (1980) that fluctuating selection in space and time maintains diversity in populations reproducing parthenogenetically. They do not imply, however, that this mechanism can maintain clonal diversity indefinitely. The low clonal richness in 1995 (Table 2, Fig. 5) and decreasing diversity during the second clonal selection era (Fig. 7) indicate that diversity will decline unless "new" clones enter from the egg bank. In the longer term (i.e., among several years), genetic diversity in facultatively parthenogenetic *Daphnia* populations is maintained by the emergence of ephippial eggs.

Importance of Resident Population Size

The period of the cycles between clonal and sexual reproduction for populations in permanent habitats, such as Long Lake, appears to depend on the population dynamics of the active population and the timing of ephippial emergence. Hatching of ephippial eggs may only be detectable or evolutionarily relevant when the active population is small. If ephippia hatch when the water column population is small, the sexually-derived immigrant clones are more likely to become established. When the active population is large, ephippial immigrants probably are not detected, because their frequencies are swamped by resident clones. Also, one would expect ex-ephippial clones to be poor competitors when the resident population is large since clones that persist in the water column are likely to be better adapted to current conditions than clones "immigrating from the past" (Templeton and Levin 1979) from the egg bank.

Microevolutionary Fits and Starts

The alternation between the importance of clonal selection during parthenogenetic reproduction, and of sexual recruitment after ephippial emergence events, underscores the

evolutionary complexity of facultative parthenogens. When individuals are reproducing parthenogenetically, clonal selection can act rapidly to change the genetic composition of active populations. The mercurial rise of a shallow water clone (C19) when trout predation was low (CS 2a - Fig. 5), and the subsequent rapid replacement of this clone by a hypolimnetic specialist (C17) when trout predation was high (CS 2b - Fig. 5), are prime examples of this.

While microevolutionary changes during clonal selection may be rapid, it is unclear whether the "winners" of clonal selection eras necessarily contribute significantly to the genetic composition of future generations. As seen in this study, ephippial recruitment events (e.g., HW1 and HW2 - Fig. 5) have the capacity to reset the evolutionary trajectory of active populations. After prolonged parthenogenesis, clonal diversity becomes depleted (Figs. 6 and 7) and a population may only consist of a few abundant clones (e.g., in 1995 and May 2000, Table 2). If sexual reproduction occurs during these circumstances, offspring of the dominant clones may have low fitness as a result of 1) the disruption of stable gene complexes responsible for the success of the common clones, and 2) the unmasking of deleterious mutations due to the inbreeding that is likely to occur in these low diversity populations (Lynch and Gabriel 1983; Innes and Hebert 1988; De Meester 1993b; De Meester and Vanoverbeke 1999). It is also possible that the risk of inbreeding when clonal diversity is low could inhibit sexual reproduction. This could result in a feedback in which less sexual reproduction, and therefore less contribution to the egg bank, occurs as a clone becomes more "successful" (i.e., increases in frequency) in the water column.

Future Directions

This study implies that zooplankton egg banks play an important role in the evolution and ecology of active populations. Until recently (Caceres 1998; Hairston et al. 1999; Cousyn et al. 2001), however, relatively few studies have directly investigated the links between the dormant egg pools and active populations.

I am presently in the midst of a project that will integrate information about the genetic compostion and magnitude of the *D. pulicaria* egg bank in Long Lake, with information presented here regarding the genetic structure and demography of the active population. This synthesis will allow for a more complete understanding of the processes that control the evolutionary dynamics of this and other cyclically parthenogenetic *Daphnia* populations.

Specifically, the dormant propagule pool of the population in the sediment will be analyzed to provide information about (1) whether the population has responded to selection imposed by predation by rainbow trout that have been annually stocked to the lake for the past 40 years, (2) how the genetic composition and diversity of the population's egg bank compares to that of the active population, and (3) what impact the emergence of the diapausing eggs has on the demography of the active population.

To determine whether or not there has been discernable evolutionary change in the *Daphnia* population in response to the fisheries management of the lake, I will attempt to hatch *Daphnia* from sediments of different ages (i.e., before and after 1961, the year that the trout stocking program began) and perform bioassays that examine characters presumed to be important for predation avoidance (e.g., size at maturity, phototactic behavior, low-oxygen tolerance).

A lack of change in these characteristics would suggest that "migration from the past" has promoted genetic constancy in the sexually-produced population. A measurable change in the characters would illustrate that the population has responded to selection despite genetic inputs from the ephippia produced during the pre-stocking era.

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Table 1. Schedule of dates that the *Daphnia* population was sampled for electrophoretic analyses, the types of samples collected, and sampling depths. Cells marked 'X' indicate that trout were collected.

DATE	WC TOW		DEEP		INTERMEDIATE		SHALLOW		TROUT	
	Depths	n	Depths	n	Depths	n	Depths	n		n
6/12/1996	22-0 m	96	22-19 m	28	-	-	11-5 m	38	-	-
6/27/1996	22-0 m	94	16-13 m	72	-	-	10-7 m	71	-	-
7/18/1996	22-0 m	96	18-15 m	96	-	-	13-10 m	93	-	-
8/1/1996	22-0 m	94	15-12 m	96	-	-	10-7 m	96	-	-
8/26/1996	22-0 m	88	14-12 m	95	-	-	11-8 m	96	-	-
10/11/1996	22-0 m	96	13-11 m	96	-	-	10-8 m	95	-	-
5/17/1997	22-0 m	90	-	-	-	-	-	-	-	-
6/16/1997	22-0 m	106	21-19 m	106	-	-	-	-	-	-
7/7/1997	22-0 m	95	19-16 m	94	-	-	-	-	X	94
8/7/1997	22-0 m	96	13-10 m	94	-	-	10-7 m	69	-	-
9/21/1997	22-0 m	96	12-11 m	96	-	-	10-8 m	94	-	-
4/22/1998	22-0 m	119	20-10 m	69	-	-	10-0 m	119	-	-
5/25/1998	22-0 m	95	22-18 m	89	13-11 m	96	11-9 m	75	X	92
6/27/1998	22-0 m	89	22-19 m	90	-	-	15-12 m	94	X	111
7/25/1998	22-0 m	93	19-16 m	93	-	-	15-13 m	176	X	107
8/22/1998	22-0 m	95	16-14 m	95	-	-	13-11 m	93	X	71
10/24/1998	22-0 m	93	17-15 m	95	-	-	-	-	-	-
4/24/1999	22-0 m	93	18-16 m	96	-	-	-	-	-	-
5/31/1999	22-0 m	96	19-16 m	96	-	-	15-12 m	69	X	107
6/27/1999	22-0 m	89	20-17 m	94	-	-	13-10 m	91	X	81
7/10/1999	22-0 m	57	18-17 m	74	-	-	16-13 m	94	X	94
8/19/1999	22-0 m	95	14-12 m	93	-	-	11-9 m	69	-	-
10/2/1999	22-0 m	95	14-12 m	93	-	-	-	-	-	-
5/17/2000	22-0 m	95	20-18 m	94	-	-	12-10 m	96	-	-

Table 2. Roster indicating the genotypic identity of clones, and which clones were observed in samples for each year. Values in the cells represent the mean percentile abundance of each clone for a given year. Dashes indicate that the clone was not collected in that year; asterisks indicate that less than 1% of the individuals sampled were of that clone type. Shaded rows indicate common clones plotted in Fig. 5. Data from 1995 are from Hembre (1996).

CLONE #	PGI	PGM	1995	1996	1997	1998	1999	2000
1	F/F	F/F	-	*	*	1.0	*	*
2	F/MF	F/F	-	-	*	*	*	-
3	F/M	F/F	-	*	*	*	*	-
4	F/S	F/F	13.4	15.7	5.5	4.5	3.8	*
5	MF/MF	F/F	-	*	*	*	*	-
6	MF/M	F/F	-	-	*	*	*	*
7	MF/S	F/F	2.2	2.3	3.8	11.6	32.9	47.2
8	M/M	F/F	-	-	-	*	-	-
9	M/S	F/F	-	*	3.3	2.3	1.5	-
10	S/S	F/F	56.3	33.6	9.7	1.2	-	-
11	F/F	F/S	-	1.0	2.8	1.9	1.1	2.1
12	F/MF	F/S	-	-	*	*	*	-
13	F/M	F/S	-	4.6	1.8	1.5	1.6	-
14	F/S	F/S	26.5	18.3	13.1	10.2	4.8	*
15	MF/MF	F/S	-	3.1	2.0	3.1	*	*
16	MF/M	F/S	-	*	3.8	3.2	1.4	*
17	MF/S	F/S	*	2.0	4.6	13.3	39.2	45.8
18	M/M	F/S	-	*	*	*	-	-
19	M/S	F/S	*	10.6	33.4	37.5	6.9	*
20	S/S	F/S	*	1.0	4.9	1.0	*	-
21	F/F	S/S	-	*	*	*	*	*
22	F/MF	S/S	-	-	*	-	*	-
23	F/M	S/S	-	*	*	1.0	*	-
24	F/S	S/S	*	2.8	7.2	3.6	*	-
25	MF/MF	S/S	-	*	*	*	-	-
26	MF/M	S/S	-	*	*	*	*	*
27	MF/S	S/S	*	-	*	*	1.1	*
28	M/M	S/S	-	*	*	-	-	-
29	M/S	S/S	-	-	*	*	*	-
30	S/S	S/S	-	*	*	*	-	-
Number of clones identified			10	23	29	28	24	13
Number of *Daphnia* assayed			1699	2023	1931	2407	1674	286

Table 3a. Frequencies of the most common clones in samples collected from the entire water column (22-0 m). For the pgi and pgm genotypes of the clones, see Table 2.

DATE	C4	C7	C10	C14	C17	C19	OTHER	N
6/12/96	0.17	0.05	0.45	0.20	0.01	0.00	0.13	96
6/27/96	0.14	0.03	0.44	0.16	0.12	0.00	0.12	94
7/18/96	0.18	0.02	0.23	0.20	0.05	0.10	0.22	96
8/1/96	0.07	0.03	0.31	0.17	0.00	0.19	0.22	94
8/26/96	0.11	0.00	0.30	0.18	0.01	0.20	0.19	88
10/11/96	0.09	0.02	0.34	0.16	0.02	0.26	0.10	96
5/17/97	0.07	0.01	0.01	0.09	0.01	0.57	0.24	90
6/16/97	0.08	0.03	0.21	0.20	0.00	0.28	0.20	106
7/7/97	0.05	0.02	0.14	0.13	0.03	0.27	0.36	95
8/7/97	0.07	0.10	0.05	0.18	0.05	0.29	0.25	96
9/21/97	0.01	0.07	0.07	0.06	0.02	0.58	0.18	94
4/22/98	0.03	0.04	0.00	0.08	0.01	0.51	0.32	119
5/25/98	0.06	0.07	0.00	0.08	0.09	0.38	0.31	95
6/27/98	0.03	0.10	0.00	0.06	0.10	0.55	0.16	89
7/25/98	0.01	0.19	0.02	0.14	0.17	0.29	0.17	93
8/22/98	0.04	0.12	0.02	0.06	0.22	0.31	0.23	95
10/24/98	0.06	0.08	0.00	0.06	0.41	0.20	0.18	93
4/24/99	0.03	0.26	0.00	0.09	0.51	0.06	0.05	93
5/31/99	0.05	0.35	0.00	0.09	0.26	0.09	0.16	96
6/27/99	0.05	0.32	0.00	0.07	0.34	0.06	0.15	89
7/10/99	0.03	0.36	0.00	0.03	0.46	0.04	0.07	57
8/19/99	0.04	0.25	0.00	0.02	0.46	0.09	0.16	95
10/2/99	0.01	0.32	0.00	0.01	0.56	0.01	0.09	95
5/17/00	0.00	0.44	0.00	0.00	0.51	0.00	0.05	95

Table 3b. Frequencies of the most common clones in samples collected from depths within the water column. For specific depths see Table 1. For the pgi and pgm genotypes of the clones, see Table 2.

DATE	DEPTH	C4	C7	C10	C14	C17	C19	OTHER	N
6/12/96	Deep	0.32	0.04	0.36	0.11	0.00	0.00	0.18	28
6/12/96	Shallow	0.29	0.05	0.16	0.21	0.08	0.00	0.21	38
6/27/96	Deep	0.10	0.01	0.56	0.24	0.01	0.00	0.08	72
6/27/96	Shallow	0.10	0.01	0.48	0.17	0.06	0.00	0.18	71
7/18/96	Deep	0.22	0.03	0.18	0.31	0.02	0.09	0.15	96
7/18/96	Shallow	0.22	0.00	0.28	0.18	0.01	0.06	0.25	93
8/1/96	Deep	0.07	0.02	0.44	0.11	0.03	0.15	0.18	96
8/1/96	Shallow	0.28	0.05	0.30	0.08	0.00	0.02	0.26	96
8/26/96	Deep	0.07	0.00	0.44	0.18	0.01	0.16	0.14	96
8/26/96	Shallow	0.21	0.03	0.24	0.16	0.01	0.14	0.22	96
10/11/96	Deep	0.05	0.01	0.48	0.13	0.01	0.22	0.10	96
10/11/96	Shallow	0.27	0.03	0.13	0.26	0.02	0.07	0.21	95
6/16/97	Deep	0.08	0.03	0.21	0.20	0.00	0.28	0.20	106
7/7/97	Deep	0.05	0.03	0.14	0.11	0.03	0.20	0.44	94
8/7/97	Deep	0.05	0.06	0.14	0.09	0.00	0.34	0.32	94
8/7/97	Shallow	0.07	0.03	0.12	0.23	0.03	0.20	0.32	69
9/21/97	Deep	0.06	0.05	0.04	0.18	0.15	0.26	0.26	96
9/21/97	Shallow	0.04	0.05	0.02	0.04	0.13	0.45	0.27	94
4/22/98	Deep	0.04	0.09	0.03	0.06	0.01	0.64	0.13	69
4/22/98	Shallow	0.05	0.08	0.02	0.05	0.03	0.62	0.16	119
5/25/98	Deep	0.03	0.13	0.01	0.02	0.09	0.46	0.25	89
5/25/98	Intermediate	0.02	0.07	0.02	0.07	0.10	0.43	0.28	96
5/25/98	Shallow	0.04	0.09	0.00	0.13	0.03	0.40	0.31	75
6/27/98	Deep	0.08	0.10	0.01	0.11	0.16	0.37	0.18	90
6/27/98	Shallow	0.04	0.11	0.00	0.07	0.06	0.49	0.22	94
7/25/98	Deep	0.04	0.23	0.02	0.13	0.21	0.19	0.17	94
7/25/98	Shallow	0.05	0.16	0.00	0.15	0.06	0.39	0.19	176
8/22/98	Deep	0.02	0.08	0.01	0.08	0.29	0.26	0.24	95
8/22/98	Shallow	0.04	0.03	0.00	0.05	0.10	0.38	0.40	93
10/24/98	Deep	0.07	0.07	0.00	0.08	0.42	0.21	0.14	95
4/24/99	Deep	0.03	0.29	0.00	0.04	0.33	0.15	0.16	96
5/31/99	Deep	0.11	0.29	0.00	0.08	0.30	0.06	0.15	96
5/31/99	Shallow	0.09	0.33	0.00	0.01	0.32	0.09	0.16	69
6/27/99	Deep	0.03	0.22	0.00	0.06	0.41	0.12	0.15	94
6/27/99	Shallow	0.04	0.43	0.00	0.05	0.25	0.09	0.13	91
7/10/99	Deep	0.01	0.34	0.00	0.04	0.50	0.08	0.03	74
7/10/99	Shallow	0.02	0.30	0.00	0.07	0.32	0.10	0.19	94
8/19/99	Deep	0.03	0.32	0.00	0.05	0.48	0.02	0.09	93
8/19/99	Shallow	0.09	0.29	0.00	0.03	0.35	0.06	0.19	69
10/2/99	Deep	0.01	0.30	0.00	0.00	0.58	0.02	0.09	93
5/17/00	Deep	0.01	0.56	0.00	0.00	0.38	0.01	0.03	94
5/17/00	Shallow	0.00	0.42	0.00	0.01	0.50	0.00	0.07	96

Table 4. Results of χ^2 tests for heterogeneity of samples collected at different depths. Clones with at least 5 individuals were included in the analysis. All other clones were pooled into a single category. For specific sampling depths, see Table 1.

DATE	SAMPLES COMPARED	DF	X^2	P-VALUE
6/12/1996	Deep, Shallow	3	4.64	0.199
6/27/1996	Deep, Shallow	3	5.19	0.159
7/18/1996	Deep, Shallow	5	7.97	0.158
8/1/1996	Deep, Shallow	5	28.6	< 0.001
8/26/1996	Deep, Shallow	5	16.6	0.011
10/11/1996	Deep, Shallow	4	50.3	<0.001
8/7/1997	Deep, Shallow	5	13.8	0.017
9/21/1997	Deep, Shallow	5	15.8	0.015
4/22/1998	Deep, Shallow	4	0.35	0.986
5/25/1998	Intermediate, Shallow	4	5.51	0.239
5/25/1998	Deep, Intermediate	4	4.50	0.343
5/25/1998	Deep, Shallow	4	10.8	0.029
6/27/1998	Deep, Shallow	4	5.86	0.210
7/25/1998	Deep, Shallow	4	26.7	< 0.001
8/22/1998	Deep, Shallow	5	21.1	< 0.001
5/31/1999	Deep, Shallow	5	4.43	0.490
6/27/1999	Deep, Shallow	4	10.1	0.039
7/10/1999	Deep, Shallow	3	12.7	0.005
8/19/1999	Deep, Shallow	2	6.11	0.047
5/17/2000	Deep, Shallow	2	4.20	0.122

Table 5a. Habitat affinities of the most common clones in 1996. 'D' indicates that the clone was proportionally more abundant in deep water; 'S' indicates that the clone was proportionally more abundant in shallow water; 'NS' indicates no significant difference in proportional abundance; and "-" indicates that sample sizes were insufficient for a statistical comparison. Significance levels are indicated by asterisks: * = p-value $\leq$ 0.1, ** = p-value $\leq$ 0.05, and *** = p-value $\leq$ 0.01.

CLONE	1996					
	6/12	6/27	7/18	8/1	8/26	10/11
4	NS	NS	NS	S***	S***	S***
10	NS	NS	NS	D*	D***	D***
14	-	NS	D**	NS	NS	S**
19	-	-	-	D***	NS	D***

Table 5b. Habitat affinities of the most common clones in 1997-1999. Codes have the same meaning as in Table 5a.

CLONE	1997		1998					1999			
	8/6	9/21	4/22	5/25	6/27	7/25	8/22	5/31	6/27	7/10	8/19
7	-	NS	NS	NS	NS	NS	NS	NS	S***	NS	NS
14	S***	D***	NS	S***	NS	NS	NS	D*	NS	NS	-
17	-	NS	-	D*	D**	D***	D***	NS	D**	D**	D*
19	D*	S***	NS	NS	S*	S***	S*	NS	NS	NS	-

Table 6. Results of χ^2 tests for heterogeneity comparing the clonal composition of *Daphnia* in trout stomachs to that of samples collected at different depths. The clonal composition of *Daphnia* in the trout stomachs consistently differed from that of the deep water samples, but did not differ significantly from the shallow water samples. For specific sampling depths see Table 1.

DATE	SAMPLES COMPARED	DF	X^2	P-VALUE
7/7/1997	Trout, Deep	7	27.5	< 0.001
5/25/1998	Trout, Deep	4	9.86	0.043
5/25/1998	Trout, Intermediate	4	3.63	0.458
5/25/1998	Trout, Shallow	4	3.20	0.524
6/27/1998	Trout, Deep	4	13.75	0.008
6/27/1998	Trout, Shallow	4	2.34	0.673
7/25/1998	Trout, Deep	4	10.15	0.038
7/25/1998	Trout, Shallow	4	5.46	0.244
8/22/1998	Trout, Deep	5	16.40	0.006
8/22/1998	Trout, Shallow	5	3.61	0.607
5/31/1999	Trout, Deep	5	14.31	0.014
5/31/1999	Trout, Shallow	3	1.75	0.625
6/27/1999	Trout, Deep	3	13.14	0.004
6/27/1999	Trout, Shallow	3	6.08	0.108
7/10/1999	Trout, Deep	4	12.15	0.016
7/10/1999	Trout, Shallow	4	8.06	0.089

Table 7. Results of χ^2 tests evaluating whether genotype frequencies deviated from Hardy-Weinberg equilibrium expectations for the two allozyme loci. Note that with the exception of the June sampling dates in 1996, and the June and July sampling dates in 1997, genotype frequencies for both loci were significantly different than Hardy-Weinberg expectations.

DATE	DF		X^2		P-VALUE	
	PGI	PGM	PGI	PGM	PGI	PGM
6/12/1996	4	2	2.31	0.61	0.679	0.739
6/27/1996	4	2	2.17	0.05	0.705	0.978
7/18/1996	6	2	28.4	12.2	< 0.001	0.002
8/1/1996	4	2	31.6	14.5	< 0.001	< 0.001
8/26/1996	5	2	51.3	19.0	< 0.001	< 0.001
10/11/1996	5	2	36.8	16.8	< 0.001	< 0.001
5/17/1997	5	2	34.4	17.7	< 0.001	< 0.001
6/16/1997	5	2	13.8	1.91	0.017	0.384
7/7/1997	5	2	6.49	1.07	0.262	0.585
8/7/1997	5	2	16.0	6.94	0.007	0.031
9/21/1997	5	2	31.0	14.7	< 0.001	< 0.001
4/22/1998	7	2	36.9	31.9	< 0.001	< 0.001
5/25/1998	7	2	25.7	12.3	< 0.001	0.002
6/27/1998	7	2	42.0	10.9	< 0.001	0.004
7/25/1998	7	2	34.0	9.82	< 0.001	0.007
8/22/1998	5	2	32.8	8.25	< 0.001	0.016
10/24/1998	7	2	33.5	7.59	< 0.001	0.023
4/24/1999	4	2	46.6	17.8	< 0.001	< 0.001
5/31/1999	7	2	42.2	4.71	< 0.001	0.095
6/27/1999	6	2	49.2	4.63	< 0.001	0.099
7/10/1999	3	2	45.7	5.53	< 0.001	0.063
8/19/1999	7	2	58.1	4.80	< 0.001	0.091
10/2/1999	3	2	51.8	6.79	< 0.001	0.034
5/17/2000	3	2	59.3	6.81	< 0.001	0.033

Figure legends

Figure 1. Population dynamics of *D. pulicaria* for 1996-1999. Sonar information was used to estimate population density on all but four dates (open circles). On those dates, population density was estimated from conventional net sampling. Error bars represent +/- 1 s.e.

Figure 2. Contour plots of temperature and dissolved oxygen for 1996-1999.

Figure 3. Sonar estimates of rainbow trout abundance for 1996-1999. Note the contrast in abundance between fall stocking (1996-1997) and spring stocking years (1998-1999). In years after fall stocking, trout were abundant over the winter, but dropped to low levels in during the summer. In spring stocking years, trout scarce over the winter (low April abundance), but were substantially more abundant during the rest of the year.

Figure 4. Annual patterns of clonal diversity for 1996-1999. Error bars are bootstrapped 95% confidence intervals.

Figure 5. Summary of the water column frequencies of common clones during the open water seasons from 12 June, 1996 to 17 May, 2000. Bracketed regions correspond to periods when the population was in Hardy-Weinberg equilibrium (HW 1 & HW 2), or had diverged from Hardy-Weinberg equilibrium and was undergoing clonal selection (CS 1, CS2a, CS 2b). Gray bars indicate winter months when the population was not sampled. Trout predation was relatively low during CS 2a, and relatively high during CS 2b (Fig. 3). Note the marked increase of clone 19, a metalimnetic specialist, during the CS 2a era (when trout predation was low), and its subsequent decline during the CS 2b era (when trout predation was high). Clone 17, a hypolimnetic specialist, replaced clone 19 as the most common clone during CS 2b.

Figure 6. The pattern of clonal richness between 20 June, 1995 and 17 May, 2000. A polynomial fit to these data explains 81% of the variance.

Figure 7. Simple regression of clonal diversity versus time during the second clonal selection era (16 June 1997 - 17 May 2000). The regression shows a significant decline in diversity over this time period.

Figure 1.

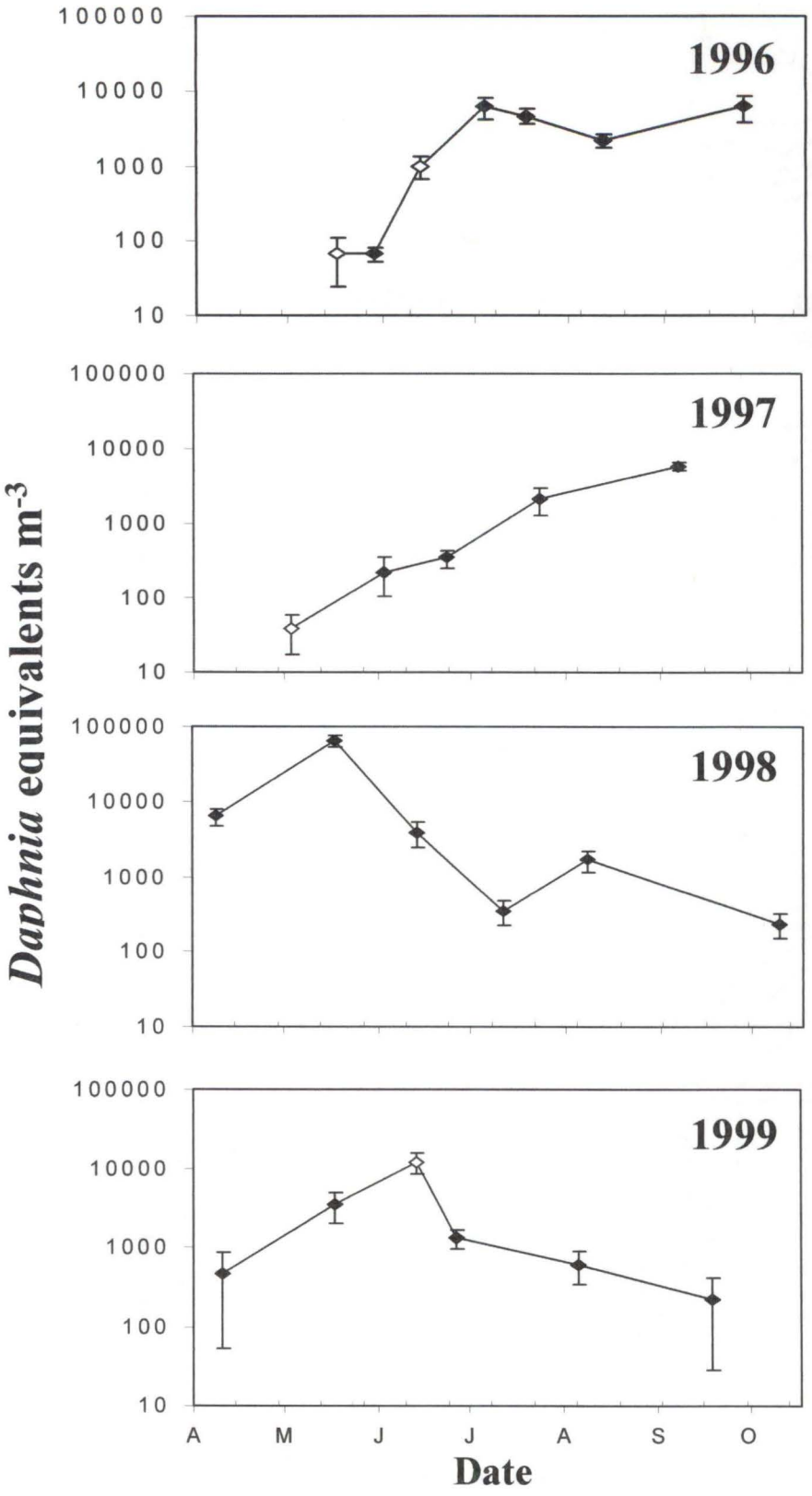

1996
1997
1998
1999
100000
10000
1000
100
10
A
M
J
J
A
S
O
Date
Daphnia equivalents m^{-3}

Figure 2.

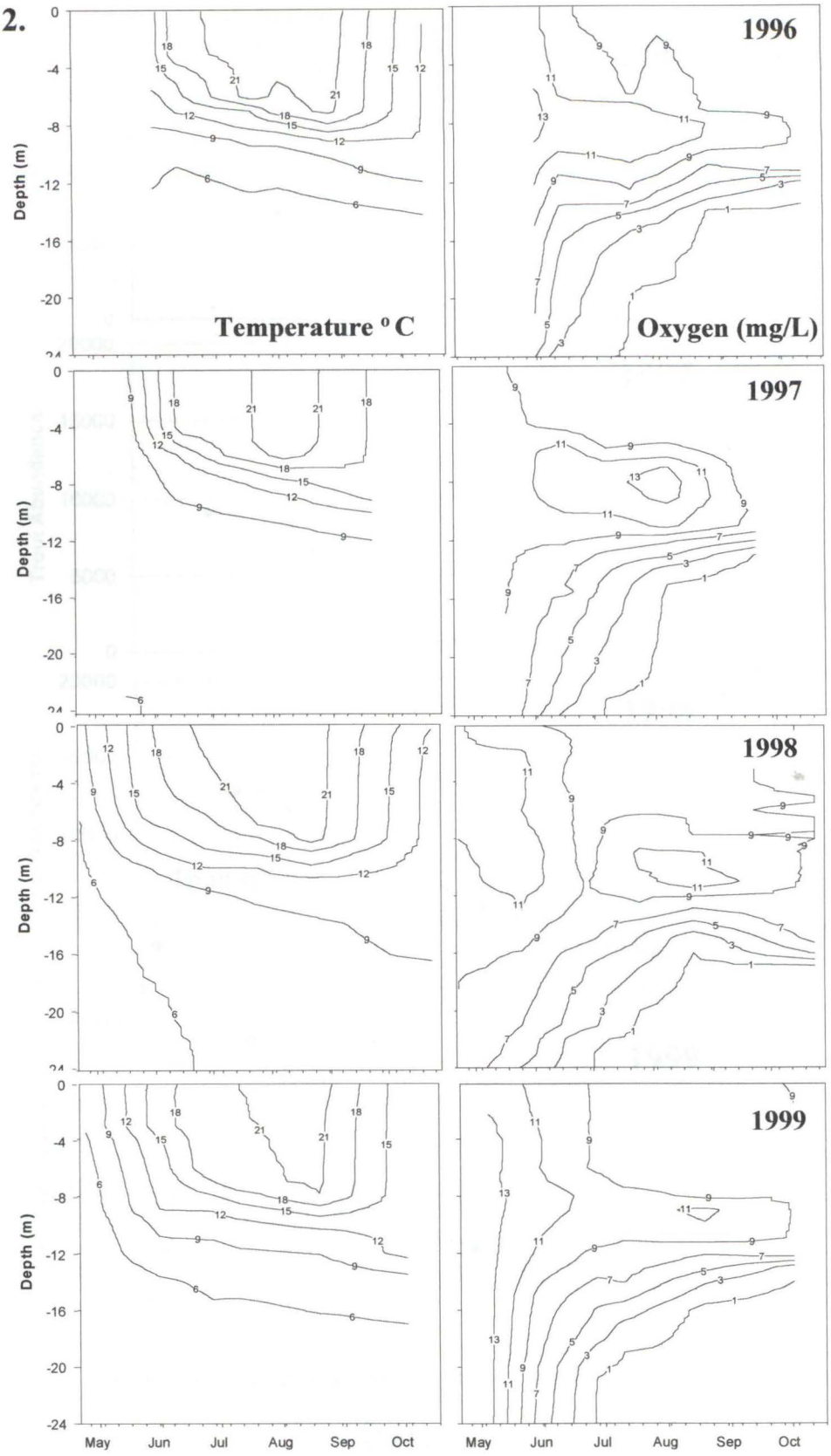

Temperature ° C
Oxygen (mg/L)
1996
1997
1998
1999
Depth (m)
0
-4
-8
-12
-16
-20
-24
May
Jun
Jul
Aug
Sep
Oct

Figure 3.

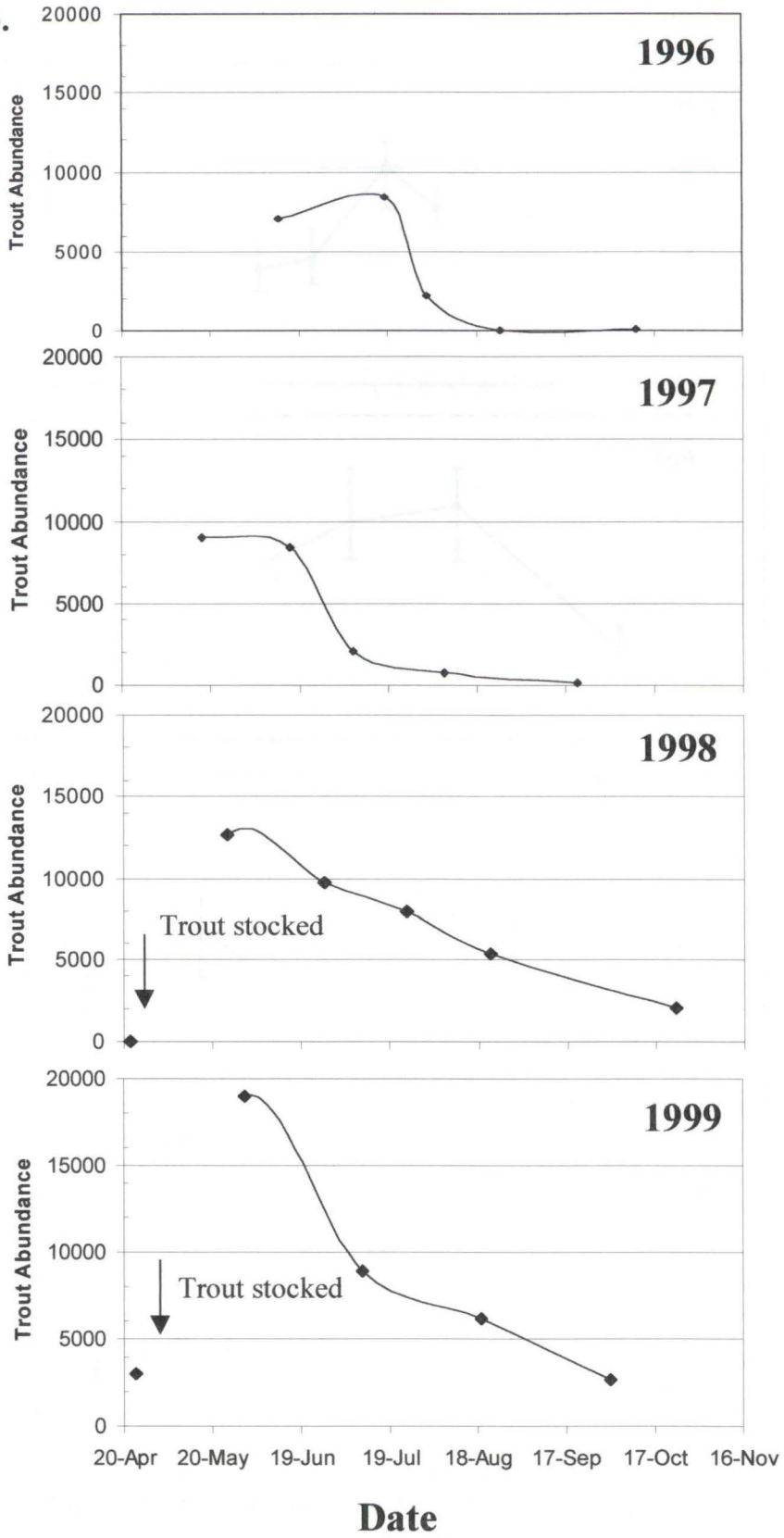
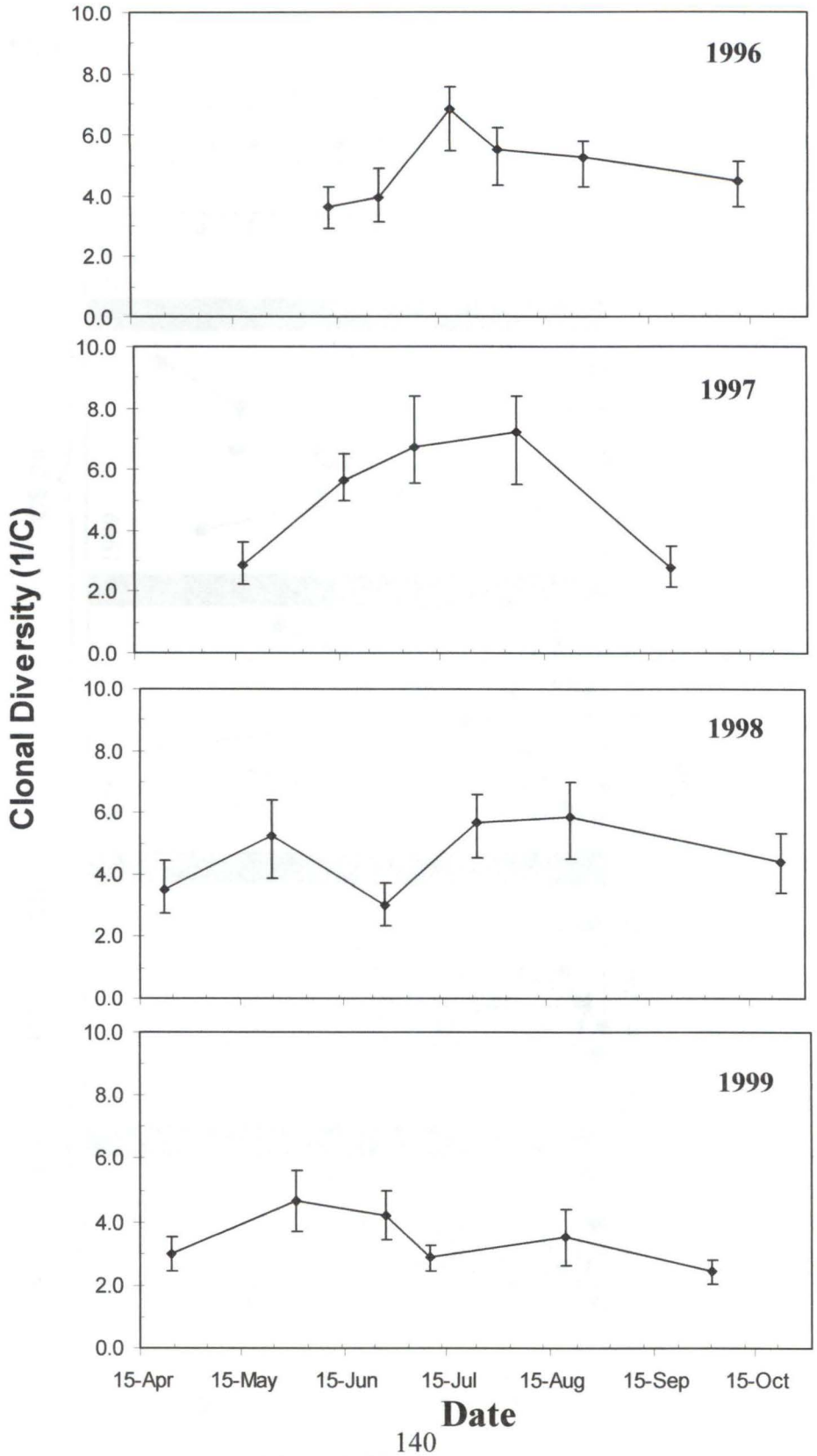

1996
1997
1998
1999
Trout Abundance
20000
15000
10000
5000
0
Trout stocked
Trout stocked
20-Apr
20-May
19-Jun
19-Jul
18-Aug
17-Sep
17-Oct
16-Nov
Date

Figure 4.


1996
1997
1998
1999
Clonal Diversity (1/C)
10.0
8.0
6.0
4.0
2.0
0.0
15-Apr
15-May
15-Jun
15-Jul
15-Aug
15-Sep
15-Oct
Date

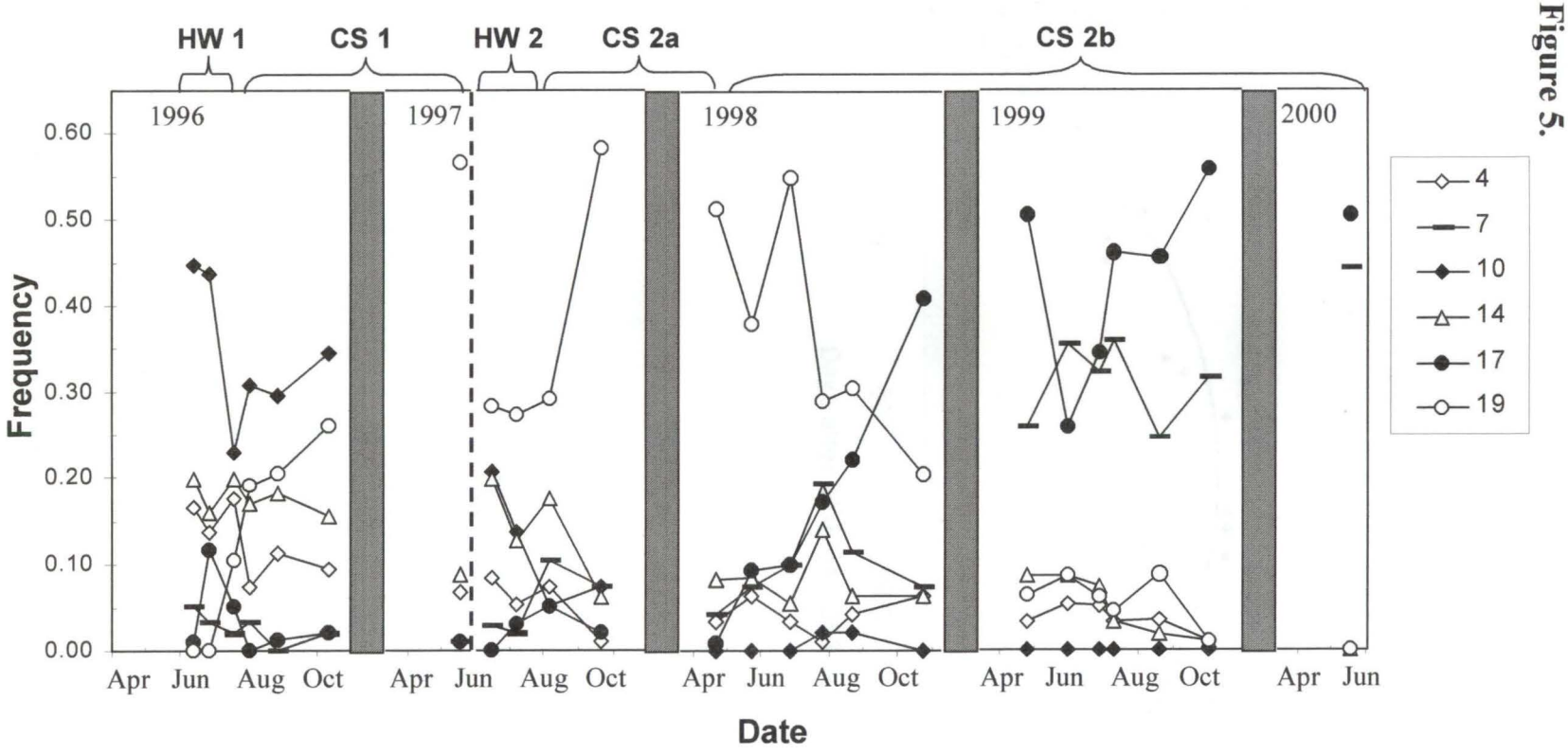

HW 1
CS 1
HW 2
CS 2a
CS 2b
1996
1997
1998
1999
2000
Frequency
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Apr Jun Aug Oct
Date
4
7
10
14
17
19

Figure 5.

Figure 6.

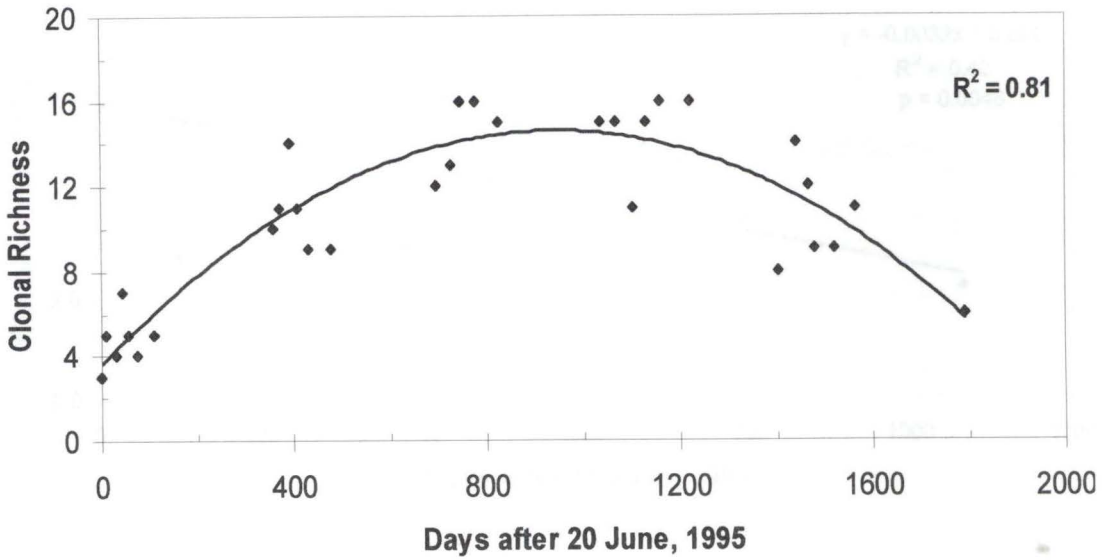
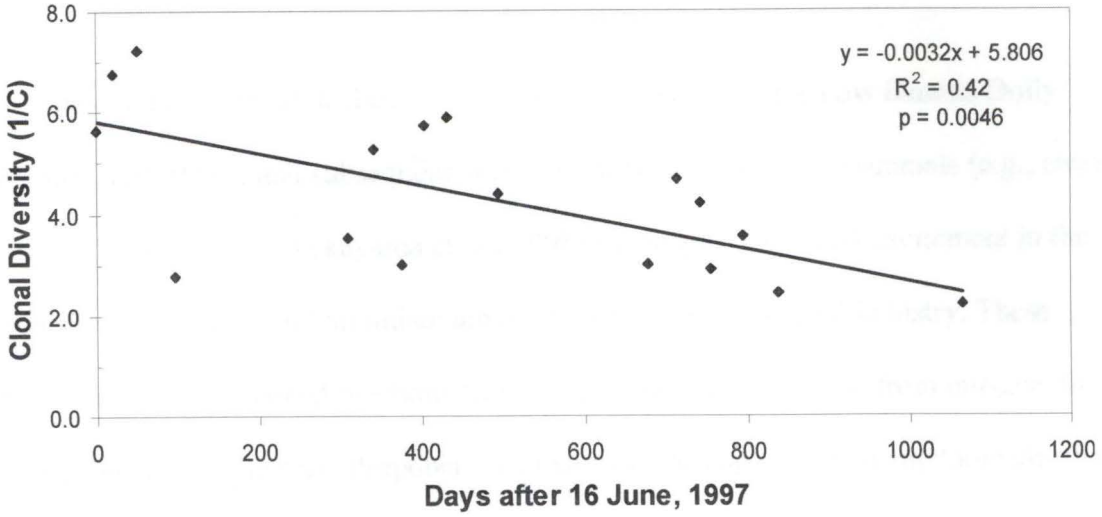

20
16
12
8
4
0
Clonal Richness
$R^2 = 0.81$
0
400
800
1200
1600
2000
Days after 20 June, 1995

Figure 7.


y = -0.0032x + 5.806
R2 = 0.42
p = 0.0046
Clonal Diversity (1/C)
8.0
6.0
4.0
2.0
0.0
0
200
400
600
800
1000
1200
Days after 16 June, 1997

Chapter 4: Evolutionary and Behavioral Consequences of Cloning

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Introduction

The cloning of an adult sheep that resulted in the birth of the now famous Dolly (Wilmut et al. 1997), and subsequent successes with cloning other mammals (e.g., cows, Kato et al. 1998; mice, Wakayama et al., 1998) has inspired awe and excitement in the scientific community, and optimism among those in the biomedical industry. These advances have also evoked reactions from the general public ranging from intrigue, to trepidation, to outright fear. Proponents of mammal cloning cite the many biomedical and economic benefits that this new technology may bring, while others question the ethics of cloning, and fear that it is only a matter of time before a human is cloned and a Pandora's Box is opened that could spawn a society akin to that of Aldous Huxley's *Brave New World.*

To most, the notion of cloning inspires far-fetched visions from science-fiction literature. In reality, though, mammals are one of the few major taxa in which some form of clonal reproduction does not naturally occur. Clonal (asexual) reproduction is widespread in nature, and its impact on the evolution, ecology, and behavior of populations has been studied extensively. The reproductive mode of a population (i.e., whether individuals in a population reproduce sexually, asexually, or both), and the degree of relatedness among individuals of a population can dramatically influence a

population's evolutionary prospects, its susceptibility to disease, and the behavioral interactions among individuals within a population.

For decades, evolutionary biologists and ecologists have debated the relative merits of asexual versus sexual reproduction and have struggled to explain why sexual reproduction is so common despite the so called "two-fold advantage" (Maynard Smith, 1971) that an individual reproducing asexually has over one reproducing sexually (Figure 1). In population genetic models with simplifying assumptions (e.g., infinite population size, no mutation, unchanging ecological conditions), asexually reproducing populations outperform sexually reproducing populations and ultimately exclude them. Only when more evolutionary and ecological realism such as mutation accumulation and finite population size (Muller 1964; Lynch et al. 1995), or changing ecological conditions (Jaenike 1978; Hamilton 1980; Hutson and Law 1981; Bell 1982) are incorporated into these genetic models do the benefits of sexual reproduction become evident.

While scholars of evolution and ecology examine the costs and benefits of sexual reproduction and the mechanisms that maintain it, behavioral ecologists focus their attention on how sexual reproduction and the resultant relatedness of individuals in a population structure the interactions among those individuals. Many of the "ultimate" explanations for behavioral phenomena observed in mammals such as cooperation in raising young or defending a territory, aggression, and choosing a mate, involve the degree of relatedness of the animals that are interacting. Kin selection is one such ultimate hypothesis that proposes that animals can increase their genetic contribution to future generations (i.e., their inclusive fitness) by aiding those with whom they share genes. For kin selection to occur, organisms must first be able to distinguish kin from

non-kin, and then adjust their behavior accordingly. Many studies have examined how the degree of relatedness among individuals affects the nature of their behavioral interactions (e.g., Holmes and Sherman 1982) and the findings of most of these studies support the idea that there is a genetic basis to kin recognition. However, confounding environmental factors (e.g., common rearing environment or maternal effects) plague these studies and leave some doubt about the relative importance of genetic versus environmental factors in controlling kin recognition. The new mammal cloning technology may allow scientists to control some of the unavoidable environmental factors that have clouded the results of previous studies and allow for a conclusive test of what influences kin recognition in mammals.

This review will not explore the ethical and sociological issues of mammal cloning, but will instead focus on evolutionary and behavioral consequences of clonal reproduction, with special attention to (1) how populations that switch between sexual and asexual reproduction differ from obligately sexual populations in the exploration of their adaptive landscapes, (2) how the reproductive mode of a population influences its long-term viability, (3) how clonally and sexually reproducing populations differ in their susceptibility to disease, and (4) how biotechnological advances in the cloning of mammals could be used to provide insight into the mechanisms that control kin recognition.

Mode of Reproduction and the Adaptive Landscape

Whether individuals in a population reproduce sexually, clonally, or are able to switch between sexual and clonal reproduction, can dramatically affect how the population responds to selective pressures. At times, the mechanics of the process of

sexual reproduction may restrict obligately sexual populations from reaching globally optimal "adaptive peaks" (Wright 1931), while those populations that can switch between sexual and asexual reproduction are released from the constraints of sexual reproduction and are able to span valleys in the adaptive landscape.

The classical viability selection model, and an adaptation of the model that incorporates generations of clonal reproduction, in the context of the human β–globin locus in West Africa, will be used to elucidate this evolutionary difference between obligately sexual populations and populations that switch between sexual to asexual reproduction.

Sickle-cell Disease and Malaria in West Africa

Viability data for the human β–globin locus in West Africa (Allison 1964) have provided a hallmark example for population geneticists, in that they provide strong empirical evidence for heterosis, and because they also suggest that the human population in this region may be "stuck" on a sub-optimal peak in the adaptive topography.

Variation at the human β–globin locus affects the incidence and severity of sickle-cell disease, and the relative susceptibility of an individual to infection by the malaria parasite *Plasmodium falciparum* (Allison 1964; Cavalli-Sforza & Bodmer 1971; Orjih et al. 1985). More than 100 alleles for the β–globin locus have been characterized, but the three most common alleles in West Africa are $Hb\beta^A$, $Hb\beta^C$, and $Hb\beta^S$ (Hartl & Clark 1989). Individuals with different combinations of these alleles are differentially affected by malaria and sickle-cell anemia. Fitness estimates of the six genotypes for these three alleles (A, S, C) are shown in Table 1.

The S allele is responsible for the sickle hemoglobin mutation. Individuals homozygous for the S allele have sickle-cell disease and the lowest fitness of the six genotypes. Homozygote individuals for the A allele do not have sickle-cell disease, but are susceptible to malaria, and also have relatively low fitness. Those who are AS heterozygotes have higher fitness than either the AA or SS homozygotes. These individuals have mild anemia, but are less susceptible to malarial infection. Carriers of the S allele have greater malaria resistance because they produce heme molecules that are toxic to the *P. falciparum* parasites (Orjih et al. 1985). These data provide solid support for heterosis as the mechanism that maintains the allele ($Hb\beta^S$) responsible for sickle-cell disease, in areas where malaria parasites are widespread.

The tidy scenario that the S allele is maintained in the population as a result of heterosis, however, is clouded by the presence of a third allele ($Hb\beta^C$). This allele was present at a relatively low frequency (0.03, compared to 0.09 for the S allele and 0.88 for the A allele) in the populations sampled by Allison (1964), despite the fact that individuals who are homozygous for the C allele have the highest fitness of the six genotypic combinations because they have malarial resistance and even milder anemia than AS heterozygotes. Under these circumstances, it is clear that population mean fitness would be maximized if the C allele were to go to fixation. If Allison's fitness estimates are accurate, there are two reasonable possibilities for why has this not occurred: (1) the C allele is a recent mutation and the population has not yet reached equilibrium, or (2) the relatively low fitness of the genotype combinations when the C allele is paired with one of the other two alleles is constraining the population from reaching the highest peak on the adaptive landscape (Hartl & Clark 1989). The following simulations will explore the

latter of these two possibilities by contrasting two models: (1) the classical viability selection model in which there is only sexual reproduction (Box 1), and (2) a model that explores the evolutionary dynamics of a population that switches from sexual reproduction to clonal (asexual) reproduction for a given number of generations (Box 2).

Box 1. Classical viability selection model

The classical viability selection model explores the microevolutionary dynamics of populations that consist of genotypes that differ in fitness, but otherwise adhere to Hardy-Weinberg equilibrium conditions (i.e., populations are diploid, randomly mating, have non-overlapping generations, an infinite population size, no migration, and no mutation).

The simulations shown in Figure 2 consider a single locus, three-allele version of this model (i.e., the β–globin locus, with alleles A, C, and S). The frequencies of the alleles A, C, and S, sum to 1, and are denoted p, q, and r respectively. Genotype frequencies are obtained as the terms of the square of the trinomial of the allele frequencies: $(p + q + r)^2$ (Table 2).

Two fundamental parameters of this model are: (1) the mean fitness ($\overline{w}$) of the population, and (2) the marginal fitness of alleles. Population mean fitness is a weighted average of the genotype frequencies and their associated relative fitness values. For this scenario:

$$\overline{w} = p^2\, wAA + q^2\, wCC + r^2\, wSS + 2pq\, wAC + 2pr\, wAS + 2rq\, wSC$$

A critical property of $\overline{w}$ in this model is that it is non-decreasing. That is, mean fitness can only increase from one generation to the next, or stay the same if the population has reached equilibrium.

The marginal fitness of an allele is a weighted average of the frequencies of genotypes that contain the allele, and their relative fitness values. The marginal fitness of the A allele (w_A) is:

$$w_A = p^2\, wAA + 2pq\, wAC + 2pr\, wAS$$

The change in an allele's frequency from one generation to the next is computed by recursion. The recursion equation for the frequency (p) of allele A is:

$$p_{t+1} = p_t\, (w_A / \overline{w})$$

In this model, an allele only increases its frequency in the next generation if its marginal fitness exceeds the population mean fitness. Since an allele's marginal fitness depends on the frequency and the fitness of the genotypes in which it occurs, certain mutations that arise that would increase the population mean fitness if they were to go to fixation may be prohibited from increasing because of the low relative fitness of some of its heterozygotic combinations. This "marginal fitness constraint" may be preventing the C allele from invading the human population in West Africa (Figure 2).

Classical Viability Selection Model Simulations

A three-dimensional adaptive topography for this scenario in which mean fitness is plotted against the allele frequencies for A and S shows the two adaptive peaks for this system (Figure 3). The highest peak is where the frequency of both the A and S alleles equals zero (i.e., fixation for the C allele). This global maximum is unattainable given the initial allele frequencies in the simulations in Figure 2. The marginal fitness constraint on the C allele restricts the population to a lower peak at A = 0.88 and S = 0.12. Under the assumptions of the classical viability selection model, it is not possible for the population to span the valley in the topography between these two peaks.

For the population in this scenario to bridge the trough in its adaptive topography, the frequency of the C allele must increase to a level that would result in the production of a sufficient frequency of the CC genotype so that the marginal fitness of the C allele would exceed the population mean fitness. Two ways that this could occur are (1) via the conditions of Sewall Wright's "Shifting Balance" theory (Wright 1931) in which population subdivision, resultant genetic drift, and interdemic selection could allow for a sufficient increase in the C allele, or (2) to incorporate generations of asexual reproduction so that the CC genotype can replicate itself, and eventually exclude the less fit genotypes. The simulation shown in Figure 4 explores the second of these possibilities and is based on an adaptation of the classical viability selection model (Box 2).

Box 2. Model incorporating asexual reproduction.

The model used to produce the simulation in Figure 4 is an adaptation of the classical viability selection model that incorporates generations of asexual reproduction. For this model, after an initial generation of sexual reproduction, the genotypes act as clones, and increase or decrease in frequency according to their fitness values. Genotype frequencies during the generations of clonal reproduction, are computed from one generation to the next (e.g., for genotype AA) by the following:

$$AA_{t+1} = (AA_t * wAA)/ \overline{w}_{clonal}$$

where,

$$\overline{w}_{clonal} = AA*wAA + AS*wAS + AC*wAC + SS*wSS + SC*wSC + CC*wCC$$

For the asexual generations of this simulation, genotype and allele frequencies are determined by the result of clonal competition, and are not limited by the mechanics of sexual reproduction. Therefore, for the human β–globin locus scenario modeled in Figure 4, the C allele is not constrained by its marginal fitness as it is when reproduction is obligately sexual (Figure 2). For this model, the CC genotype rapidly spreads and the C allele goes to fixation.

Clonal Reproduction Model Simulations

The simulation in Figure 4 illustrates how rapidly a "good genotype" can come to predominate in a population that reproduces clonally. One can visualize that depending on the relative fitness of the different genotypes; it may only take a small number of asexual generations between sexual generations for an allele in a successful genotype to increase its frequency such that it exceeds the critical marginal fitness threshold. Once beyond this threshold, the salutary allele would go to fixation even if the population were reproducing sexually. For populations that switch between sexual and asexual reproduction, there likely is an optimal number of asexual generations between sexual generations that maximizes the benefits of the two reproductive modes.

Too many asexual generations interjected between bouts of sexual mixis could make a population susceptible to several ills. During the asexual phase, these negative effects could include (1) mutation accumulation and the effects of Muller's Ratchet (Muller 1964), and (2) a higher incidence of disease (Hamilton 1980). Since the clonal diversity of populations reproducing asexually typically decreases over time (King 1977), sexual mixis may increase the likelihood of matings between individuals of the same clonal lineage. These intra-clonal matings could result in another ill to the population – inbreeding depression.

Conversely, too few asexual generations between sexual generations may not allow adequate time for beneficial rare alleles to propagate to a frequency necessary to become relatively safe from loss by random genetic drift.

While incorporating generations of clonal reproduction to a human population is neither a technological possibility at this time nor an ethical one, the pattern of switching

between sexual and asexual reproduction is utilized by a wide variety of taxa in nature (e.g., Cladoceran crustaceans such as *Daphnia* (Figure 5), aphids, protists such as *Volvox*, many plant species). This flexibility may allow populations of these taxa to explore their adaptive topographies more dynamically in the short-term and to reach population mean fitness maxima that would be unattainable if sex were the sole means of reproduction.

Long-term Consequences of Clonal Reproduction

The reproductive mode of a population and the genetic consequences that accompany it not only influence a population's adaptability to its current conditions, but also its responsiveness to change. The relatively simple model in the previous example illustrates how clonal reproduction can benefit a population by allowing it to more dynamically explore its current adaptive landscape than could an obligately sexual population. If a population's microevolutionary milieu is fixed and genotype fitness values do not change, it is difficult to understand why sexual reproduction would ever be favorable. In reality, though, mutations, changes in ecological interactions among species, and changing environmental conditions ensure that genotypic fitnesses and a population's adaptive landscape are not static. Two of the primary factors thought to offset the two-fold cost of sexual reproduction (Maynard Smith 1971) are the ability of sexual populations (1) to purge deleterious mutations and (2) to produce genetically variable offspring that allow them to respond to changing conditions.

Mutation Accumulation & Muller's Ratchet

Models that account for the reality that populations are finite and that mutations occur reveal one of the significant advantages of sexual reproduction - that sexual recombination allows populations to purge deleterious mutations. In asexual populations,

an individual can never produce offspring with fewer deleterious mutations than it carries itself. Assuming that back mutations are rare and that mutations are, on average, deleterious, asexual lineages inevitably accumulate mutations and become less fit with each successive generation. This mechanism, known as Muller's ratchet (Muller 1964) poses a serious risk of extinction for asexual populations (Maynard Smith 1978; Charlesworth et al. 1993; Lynch et al. 1995) and may be largely responsible for the long-term instability of asexual species. Bdelloid rotifers are the only known exception to the rule that asexual lineages are less evolutionarily stable than are sexual species. The ability of bdelloid rotifers to produce a dormant stage when conditions are poor, and their exceptional dispersal ability may have allowed them to avoid extinction (Vrijenhoek 1998).

In sexual populations, recombination slows down the ratchet by allowing for the production of progeny chromosomes that incorporate the best portions of the two parental chromosomes. In essence, sexual recombination can act as DNA repair at the population level. Sexual populations, though, are not immune to the effects of Muller's ratchet. In relatively small populations, mutation, genetic drift, and selection can interact and lead to the eventual extinction of populations as a result of "mutational meltdowns" (Lynch et al. 1995). While mutational meltdowns are a legitimate risk for small sexual populations, it is very improbable that deleterious mutations will fix in relatively large sexual populations (i.e. effective population size greater than a few hundred individuals) (Kimura 1962).

The extent to which populations that switch between sexual and asexual reproduction will be affected by Muller's ratchet is likely to depend on (1) the absolute

population size, (2) the number of asexual generations between sexual recombination events, and (3) the strength of selection during the asexual phase. Absolute population size is important because, even in a strictly parthenogenetic population, there is a threshold population size above which a population is relatively safe from the risk of a mutational meltdown ($N > 10^5$, Lynch et al. 1995). The number of asexual generations between sexual generations is important because, coupled with the strength of selection, it will largely determine the clonal diversity of the population and thus the population's potential to purge deleterious mutations during sexual recombination. Long periods of asexual reproduction between sexual generations increases the probability that clonal lineages will accumulate mutations, decreases the clonal diversity of the population, and ultimately limits the effectiveness of sexual recombination to purge deleterious mutations.

Responding to Co-evolving Parasites – The Red Queen Hypothesis

The second major factor thought to offset the cost of sexual reproduction is the sexual production of genetically variable populations of offspring that evolve in response to co-evolving natural enemies. Parasites are under strong selection to infect the most common host genotypes, so in host-parasite interactions, novel or rare genotypes have an intrinsic advantage and may be selected for (Lively 1996). Eventually, these previously rare genotypes increase in frequency and parasites may co-evolve to adapt to the changes in their hosts. Sexual reproduction allows host populations to respond to the counter-attacks of their parasites by continually producing novel genotypes that are less susceptible to infection. The inability of asexual offspring to escape the antagonistic advances of co-evolving parasites is a major disadvantage to asexuality. A growing body

of theoretical and empirical evidence supports the notion that time-lagged, frequency dependent selection by parasites on their hosts produces sustained oscillations in host and parasite gene frequencies. These sustained oscillations maintain genetic variation (Hutson & Law 1981) and may lead to selection for sexual reproduction (Jaenike 1978; Hamilton 1980; Bell 1982). Because of the treadmill-like oscillations in host and parasite allele frequencies, this idea has come to be known as the *Red Queen hypothesis* in reference to the line in Lewis Carroll's Through the looking glass and what Alice found there by the Red Queen to Alice:

"Now, here, you see, it takes all the running you can do to keep in the same place."

Empirical Support for the Red Queen Hypothesis

A central assumption to the Red Queen hypothesis is that parasites differentially attack the most common genotype. Empirical studies of a variety of systems support the validity of this assumption. Studies of agricultural systems have shown that fungal pathogens attack cereal monocultures more severely than mixed cultures (Brown 1994) or wild-growing relatives of the cultivated crop (Apple 1977). Studies examining parasite loads in animal populations (lizards, Moritz et al. 1991; minnows, Lively et al. 1990) with co-occurring sexual and parthenogenetic individuals also support the Red Queen assumption that parasites differentially infect the most common genotype.

Another important condition of the Red Queen hypothesis is that there is a time-lag in the selection by parasites against the common genotypes. This type of selection allows for the oscillations in host and parasite genotype frequencies that are proposed to select for cross-fertilization as a strategy for the genotypic diversification of offspring (Jaenicke 1978; Hamilton 1980; Bell 1982). The hypothesis that time-lagged, frequency-dependent

selection acts to maintain genetic diversity has been difficult to examine empirically, but the findings of a recent study of the dynamics of a clonal population of freshwater snails (*Potamopygrus antipodarum*) and its trematode parasites supports this hypothesis (Dybdahl and Lively 1998).

To evaluate this hypothesis, the frequencies of the host clones and the dominant trematode parasite (*Microphallus* sp.) were determined over a five-year period. Also, the susceptibility of infection of the rare and common snail clones was examined in laboratory experiments. The results of the study are consistent with the predictions of the Red Queen hypothesis in that the parasites responded to the common snail clones in a time-lagged manner, and the rare clones were less susceptible to infection than the common clones in laboratory experiments.

Application of Cloning Technology to the Study of Animal Behavior

While the preceding sections of this review have explored some of the evolutionary and ecological implications of clonal reproduction, this section will speculate about how the new mammal cloning technology could be applied to evaluate one of the long-standing questions in animal behavior: How do animals recognize their kin?

Distinguishing kin from non-kin is critical for organisms when deciding with whom to behave cooperatively, and when choosing mates. An organism enhances its inclusive fitness (Hamilton 1964) when it cooperates with relatives in raising young (Emlen and Wege 1988), defending a territory (Sherman 1981), alarm calling (Hoogland 1983), or any other endeavor that aids the survival and reproduction of one's kin. When choosing a mate, determining relatedness is important because it allows an individual to avoid inbreeding (Bateson 1983) and to enhance the genetic quality of its offspring.

While it is accepted that kin recognition occurs in nature and that it affects many of the behavioral interactions among individuals of the same species, the mechanisms that control it are still not entirely clear. One school of thought holds that organisms associate cues (e.g., odors) from their rearing environment with kin, and use these learned cues to assess the relatedness of individuals that they encounter. Others contend that the recognition of relatives is genetically based and does not require learning particular environmental cues associated with a common rearing environment.

Environmental Association and Kin Recognition

Considerable evidence suggests that animals associate cues from their rearing environment to identify kin. A study examining the huddling behavior in spiny mice (Porter et al. 1981) shows that, if given a choice, the mice prefer to huddle with littermates as opposed to unfamiliar individuals. In nature this results in siblings huddling together. However, when litters of non-siblings cared for by a single mother were created experimentally, the unrelated littermates preferred to huddle with each other more than with their true siblings.

Factors that affect kin recognition in Belding's ground squirrels (Holmes and Sherman 1982) further illustrate the importance of environmental associations, but also highlight the complexity of the subject of kin recognition. For this experiment, pregnant females were captured and their pups were used to create four kinds of experimental rearing groups: (1) siblings reared by the same mother (their own or a foster mother), (2) siblings reared apart by different mothers, (3) non-siblings reared as a single litter, and (4) non-siblings reared apart. When older, the animals were placed in pairs in arenas and their interactions were observed. The results showed that, irrespective of relatedness,

animals reared together rarely fought, thus supporting the notion that animals learn to identify their kin. While rearing environment was the most important factor affecting the animals' aggressiveness in pair encounters, the results also showed that among females reared apart, sisters were less aggressive to each other than were unrelated females. The authors suggest two mechanisms to account for this result. One is that sisters reared apart may have been able to recognize each other based on cues learned from a common prenatal experience in their mother's uterus. A second possibility is that the females used what they term "phenotype matching" to distinguish kin from non-kin. Phenotype matching is the idea that individuals act altruistically toward those that are phenotypically similar in some way (e.g., have a similar odor). This mechanism blurs the line between the importance of learning OR genes in kin recognition. It requires that the animal learns a cue to distinguish related from unrelated individuals, but instead of stemming from a common environment the learned cue reflects a genetic identity.

Non-Environmental Control of Kin Recognition

There is strong evidence supporting the notion that animals use cues learned from their rearing environment to determine with whom to behave cooperatively. In nature, this usually results in cooperation among kin. However, others have proposed that the mechanism for kin recognition is independent of environmental factors. Two of the non-environmental mechanisms proposed to allow organisms to recognize their kin are the "green beard effect" (Dawkins 1976) and phenotype matching (Holmes and Sherman 1982).

The green beard effect is a wholly genetic mechanism that does not require learning. Dawkins proposes that organisms may have "recognition alleles" that express their

effects phenotypically (e.g., as a green beard). The phenotypic signal then allows individuals bearing these particular alleles to recognize them in others and causes the bearers of the alleles to behave altruistically toward others with the recognizable phenotypic effect. If such recognition alleles exist, they could provide a means for kin recognition without learning. While an interesting theoretical possibility, there is little or no empirical support for this mechanism.

Phenotype matching is similar to the green beard effect in that the basis for distinguishing kin from non-kin is genetic. However, unlike the green beard effect, kin recognition via phenotype matching requires that organisms learn their own phenotype and compare it with those of others to assess relatedness. For phenotype matching to be a reliable means of assessing kinship, organisms must learn a cue that characterizes their genetic identity and is sufficiently variable among individuals in a population such that there is a means to distinguish similar and dissimilar individuals. The production of different chemical cues or odors as a result of genetic variation in the major histocompatibility complex (MHC) may be the phenotypic cue that allows many animals to assess the relatedness of conspecifics.

Operation of the MHC Matching System

The MHC is a matching system by which the immune system discriminates self from non-self (Brown and Eklund 1994). In most cases, "non-self" is a disease organism. It may also be possible, however, that organisms use this system to detect the level of genetic similarity between themselves and others.

At the cellular level, MHC genes code for cell-surface glycoproteins that play a vital role in immune reactions. These glycoproteins are basket-shaped and can "grasp" small

peptides that are presented to them by the cells. If the peptide presented by the cell is evaluated as a "self-peptide" it is left alone. However, if a virus infects the cell, MHC-coded proteolytic machinery (Goldberg & Rock 1992) inside the cell puts a small peptide from the virus into the basket of the glycoprotein. The peptide is then recognized as a "non-self peptide" and cytotoxic T cells alerted to the presence of the virus proceed to kill the infected cell.

While relatively few MHC loci operate in cell recognition for a given species, each locus typically has many alleles so that virtually no two individuals have identical MHC genotypes (except identical twins and organisms in highly inbred populations). The uniqueness of an organism's MHC genotype is critical to distinguishing self from non-self in the context of an individual's immune system. Research has also shown that this uniqueness may be important at the behavioral level. The MHC can produce phenotypic signals, such as body odors and odors in urine, that some animals can detect. This may allow them to avoid mating with close relatives, and to determine with whom to cooperate.

Empirical Support for MHC Matching

Empirical support for the importance of the MHC in kin recognition comes from studies of a variety of animals ranging from protochordate tunicates (Grosberg & Quinn 1986), to mammals such as mice and humans.

Genes in the MHC of house mice (*Mus musculus*) affect body odor. Mice then utilize the phenotypic odor signals to ascertain the relatedness of individuals they encounter. Several studies (Potts et al. 1991; Eklund 1997; Penn and Potts 1998) show that mice use these odor cues to avoid mating with MHC-similar individuals. In addition to using the

odor cues to avoid inbreeding, there is also evidence that female mice are more likely to nest communally with other females with similar odors, such as sisters (Manning et al. 1992a). Communal nesting of sisters presumably enhances their inclusive fitness because they aid in rearing their nieces and nephews as well as their own offspring.

The evidence for MHC control of kin recognition in humans is more tenuous, but the results of several studies suggest that genetic variation in the MHC in humans affects mate choice. In two of these studies (Wedekind et al. 1995; Wedekind and Furi 1997), men and women were asked to rate the attractiveness of the odors of T-shirts worn by MHC similar and dissimilar individuals. Results showed that both men and women preferred the odor of MHC dissimilar individuals. While detractors have questioned some of the methodology in these studies and whether or not T-shirt odor preference is an accurate proxy of mate choice, the results do support the notion that odor cues produced by MHC genes allow humans to distinguish related from unrelated individuals.

A study of a population of Hutterites provides additional evidence of MHC-dependent mating preferences in humans (Ober et al. 1997). An analysis of genetic data shows that this population has relatively low MHC diversity. The low genetic diversity of the MHC is likely a result of the genetic bottleneck that occurred when about 400 Hutterites migrated to North America from Europe in the late 1800s. In spite of this low MHC diversity, however, there is a deficit of MHC homozygotes at birth (Kostyu et al. 1993). Homozygote deficiency suggests either that individuals are mating disassortatively, or that there is abortional selection against MHC homozygotes. Further support for the importance of the MHC comes from a statistical analysis of the genetics of the population that shows that Hutterite couples are less likely to share MHC

haplotypes than by chance, even after statistically controlling for factors such as nonrandom mating patterns among colony lineages, and close inbreeding taboos.

Using Mammal Cloning Technology to Test Kin Recognition Mechanisms

Mechanistic studies of kin recognition in mammals provide evidence that animals distinguish kin from non-kin (1) on the basis of cues learned from a common rearing environment (e.g., huddling behavior in spiny mice), (2) by matching phenotypic characters linked to genetic differences (e.g., recognition that relies on identifying odors controlled by the MHC), or (3) by some combination of the two (e.g., kin recognition in Belding's ground squirrels). While they have contributed greatly to the understanding of how organisms recognize their kin, these studies have been unable to control certain factors that potentially confound the interpretation of their results.

The methodology for many of these studies is to separate siblings at birth, rear them by foster mothers, and then later reintroduce the unfamiliar siblings to each other to see if they are more likely to treat their true siblings or those they were reared with as kin. While the intention of these studies is to compare the effect of environmental versus genetic factors in kin recognition, the prenatal experience of the siblings is not controlled. Therefore, one cannot conclude with certainty whether non-familiar sibling recognition is a result of maternal effects or if it is genetically based.

Laboratory studies examining mating preferences in mice have also been criticized because most of the work has been on highly inbred lab mice (Penn and Potts 1999). Extrapolating results from studies using inbred mice to mice from wild populations could be misleading because (1) the MHC diversity in inbred strains is likely to be much less

than that of wild populations, and (2) it is possible that inbreeding avoidance behaviors are selected against during domestication (Manning et al. 1992b).

An experiment using the new mammal cloning technology could allow for a thorough test of the relative importance of environmental and genetically-based cues in kin recognition without the need for inbred strains, and while controlling for maternal effects.

Rationale and Experimental Design

Since the birth of Dolly the sheep in 1996 (Wilmut et al. 1997), other mammal species have been successfully cloned (e.g., cows, Kato et al. 1998; and mice, Wakayama et al. 1998) in a similar manner (Figure 6). By utilizing this technology, behavioral biologists could gain tremendous insight into the mechanisms that control kin recognition because it is possible to design an experiment that would allow for the evaluation of the relative importance of three important factors (1) relatedness, (2) rearing environment, and (3) maternal effects.

Of the mammals that have been successfully cloned to date, mice are the logical choice for an experimental organism. They are small and easy to maintain in the laboratory, and a single litter can potentially produce multiple clones. Past studies examining kin recognition in mice have usually used inbred laboratory strains. For this experiment, though, one could sample wild populations of house mice (*Mus musculus*) to obtain animals to be cloned.

The basic methodology for the experiment would be similar to several other studies that have examined the factors that control kin recognition. Some behavioral response related to kin recognition would be evaluated when animals of varying relatedness and

familiarity are allowed to interact. The behavioral response to be assessed could be mating preference, or the level of aggression or cooperation (e.g., nesting, or huddling) displayed between the actors. While similar in basic design to previous studies, two important differences would set an experiment using mammal cloning technology apart from its predecessors.

One difference is that one could produce mice of varying levels of relatedness ranging from unrelated to genetically identical clones. In past studies investigators commonly observed the interactions among siblings and half-siblings, but it was not possible to produce genetically identical individuals. Expanding the range of relatedness of the test subjects would allow one to better evaluate whether the degree of relatedness has a significant effect on the nature of the behavioral interaction studied. A second important improvement is that one could control for maternal effects. That is, individuals that are the genetic equivalent of siblings or half-siblings would not have to necessarily share a common pre-natal environment. Because the cloned embryos are carried to term by foster mothers, siblings could be cloned and experience different pre-natal environments. Controlling for the potentially confounding effects of a shared pre-birth experience would allow for a true separation of genetic and environmental influences.

A full-factorial ANOVA experiment examining the effect of (1) relatedness, (2) rearing environment, and (3) maternal environment on the nature of behavioral interactions between individuals could provide tremendous insight into the relative importance of these factors in kin recognition in mammals. At a minimum, one would want to examine the interaction between individuals of three levels of relatedness (clones, full siblings, and unrelated individuals), two levels of rearing environment (common or

different), and two levels of maternal effect (common foster mother, or different foster mother).

Given that the success rate for the cloning procedure in mice ranges from 1 in 40 to 1 in 80 survivors for every embryo implanted (Wakayama et al. 1998), the experiment proposed here would currently be extremely labor and technology intensive. However, it is likely that as the procedure is fine-tuned, the success rate will improve and such an experiment could become feasible.

Summary & Synthesis

Cloning is a term new to the popular vernacular and a subject in its relative infancy in mammalian genetic and biotechnological research. In nature, though, clonal reproduction is common and its impact on the evolution and ecology of populations has been studied for decades.

From a strictly reproductive standpoint, an organism that reproduces clonally has an advantage over a sexually reproducing organism because it does not spend any of its reproductive resources on males. This "two-fold" advantage provides asexual populations with a short-term benefit that allows them to rapidly displace sexual populations in models (Figure 1) that do not account for certain genetic and ecological realities.

The short-term benefit of clonal reproduction, though, is counteracted by costs that are realized when models incorporate more realism and explore the longer-term ramifications of asexual reproduction. The effect of mutation accumulation on clones, and the coevolution of disease organisms are the two primary factors that negatively affect clonal populations. Unlike sexual populations, clonal populations are unable to purge deleterious mutations and are likely to succumb to the effect of Muller's ratchet

unless they maintain a large population size (> 10^5 individuals, Kimura 1962). The low genetic diversity of clonal populations also makes them vulnerable to the effects of disease organisms that adapt to attack common host genotypes. While sexual populations that continually produce genetically novel offspring can keep parasites at bay to some extent (Red Queen hypothesis), clonal populations, whose sole means of new genetic variation is mutation, are relatively fixed targets to parasites.

A Little Bit of Sex (or Asex) Goes a Long Way

Most theoretical work examining the relative merits of asexual and sexual reproduction compares obligately sexual to obligately asexual populations. These studies typically show an advantage to asexual reproduction when the conditions of the models are relatively simple, and an advantage to sexual reproduction when models incorporate more realism (Hurst and Peck 1996). Relatively few studies (e.g., Kondrashov 1985; Peck 1994), explore the evolutionary expectations for populations that reproduce both sexually and asexually. These populations have the potential to exploit the virtues of both reproductive modes, without incurring their associated costs.

Hurst and Peck (1996) state that "a little sex goes a long way" and that an ideal reproductive strategy for a population may be to reproduce asexually most of the time and periodically incorporate a generation of sexual reproduction. Interjecting one or a few generations of sexual reproduction is an efficient means of increasing the incorporation of beneficial homozygotes and uniting advantageous alleles at different loci (Green & Noakes 1995). Incorporating generations of sexual reproduction also enables a population to purge deleterious mutations and counteract the action of Muller's ratchet.

The simulations examining the sickle-cell/malaria scenario in West Africa (Figs. 2-4) reveal another benefit to populations that switch between sexual and asexual reproduction. This example suggests that "a little cloning goes a long way". By switching from sexual to clonal reproduction for several generations, the population modeled in this simulation reached the optimal peak in its adaptive topography (Figure 4) that would have been unattainable if the population reproduced only sexually. Incorporating a number of asexual generations between sexual generations can allow for the spread of a rare beneficial genotype that might otherwise not invade due to marginal fitness constraints.

The Red Queen and Kin Recognition

In addition to being a mechanism proposed to maintain genetic diversity in host populations and to select for cross fertilization, negative frequency dependent selection by parasites on their hosts (a central feature of the Red Queen hypothesis) is thought to play a vital role in MHC-mediated kin recognition. The high allelic diversity of MHC loci is critical to their use and reliability as an indicator of relatedness (Penn and Potts 1999). Without this allelic diversity, the MHC-controlled odor cues produced by individuals would not be dependable indicators of genetic identity. Because the MHC also plays a critical role in the immune system, it has been suggested that negative frequency dependent selection (i.e., selection favoring rare alleles) by parasites is the mechanism that maintains the high genetic diversity of the MHC (Potts and Wakeland 1990).

Mammal Cloning Applications

In addition to providing insight into the relative importance of the various mechanisms thought to allow animals to identify their kin, mammal-cloning technology could also prove valuable in conservation efforts. One potential use for this technology is to clone individuals from endangered or threatened species. Biologists in China are actively pursuing this strategy in an effort to save the endangered giant panda from extinction (Cohen 1997).

Limitations and Concerns for Mammal Cloning

From a scientific standpoint, there are several obstacles to the use of this new technology. One is that, currently, less than 2 percent of the implanted embryos survive to term (Wakayama et al. 1998). Unless the success rate improves, it is unlikely that a large controlled experiment to evaluate the mechanisms of kin recognition would be feasible. Another problem that has come to light that would hinder the use of this technology for mammalian conservation is that the cloned animals appear to be experiencing premature aging (Sheils et al. 1999). The lengths of the telomeres in sheep cloned at the Roslin Institute are shorter than in sheep born by natural means. Telomeres are pieces of DNA found on the ends of chromosomes that get smaller with repeated DNA replication and are thought to signal aging and death in cells. Dolly, at age two, had telomeres the length of those of a six to eight year old sheep. It is not yet clear if the shortened telomeres correspond to the physiological ages of the animals, but this research suggests that animals cloned with the somatic cells of older animals may, in effect, inherit their age as well as their genes. The relative inefficiency of the cloning process and the possibility that cloned mammals will prematurely age are two of the main technical

concerns that could limit the use of this process for behavioral studies and in conservation.

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Table 1. Occurrence of hemoglobin A, S, and C genotypes and fitness estimates based on Hardy-Weinberg expectations for 72 West African populations.

Genotype	**AA**	**SS**	**CC**	**AS**	**AC**	**SC**	**Total**
Observed count	25,374	67	108	5,482	1,737	130	32,898
Expected Count	25,615.5	306.87	74.69	4,967.2	1,768.6	165.01	32,898
Fitness[a]	0.991	0.218	1.446	1.104	0.982	0.788	---------
Relative Fitnesses[b]	0.686	0.151	1	0.763	0.679	0.545	---------

a: Fitness values calculated from the ratio of the observed counts to those expected under Hardy-Weinberg equilibrium.
b: Relative fitnesses standardized by taking wCC = 1.

Table 2. Frequencies for the six genotypes after sexual reproduction.

Genotype	**Genotype Frequency**
AA	p^2
CC	q^2
SS	r^2
AC	2pq
AS	2pr
SC	2rq

Figure Legends

Figure 1. This figure (adapted with permission from Lively, 1996) shows the number of generations it takes for a clone beginning with one individual to replace a sexual population with K individuals. The end point of each curve represents extinction of the sexual population. The rapid displacement of the sexual subpopulations by the clonally reproducing subpopulations in these simulations illustrates the demographic cost that sexual populations incur by producing males. See Lively, 1996 for details on model used to generate these curves.

Figure 2. This figure illustrates how allele (panel a) and genotype (panel b) frequencies for the human β–globin locus change with respect to the population mean fitness (wbar) and the marginal fitness values for the three alleles (panel c) under the conditions of the classical viability selection model. The relative fitness values and initial allele frequencies used in this simulation are from Table 1. Note that in this obligately sexual scenario the C allele is unable to increase in frequency despite the fact that the CC genotype has the highest fitness. The mechanics of sexual reproduction constrain the marginal fitness of the C allele from ever exceeding the population mean fitness in this simulation.

Figure 3. This figure shows the adaptive topography for the human β–globin locus example. When the population is limited to sexual reproduction, and the other assumptions of the classical viability model are upheld, the marginal fitness of the C allele constrains the population to the sub-optimal adaptive peak.

Figure 4. This figure shows what happens when the population switches to clonal reproduction. In this case, the C allele invades and goes to fixation (panel a) as a result of the competitive superiority of the CC genotype (panel b). During the generations of clonal reproduction in this scenario, the C allele is not encumbered by its marginal fitness and the population reaches the optimal peak in its adaptive topography (i.e., fixation of the C allele).

Figure 5. Many species in the genus *Daphnia* (water fleas) are cyclically parthogenetic. That is, individuals reproduce parthogenetically (clonally) for a number of generations and then may reproduce sexually. The top image of this figure (a) shows a female carrying a brood of several parthenogenetic eggs. These eggs most often develop into females, but under certain conditions may develop into males. The image below (b) shows a female with an ephippium. Ephippia are capsules that contain two haploid eggs that may be fertilized by males. Successfully fertilized ephippia undergo a period of diapause before the sexually-produced *Daphnia* hatch.

Photos: R.O. Megard, University of Minnesota – Twin Cities.

Figure 6. This figure illustrates the methodology by which mammals may be cloned (adapted with permission from Wakayama et al. 1998, Nature ©).

Figure 1.

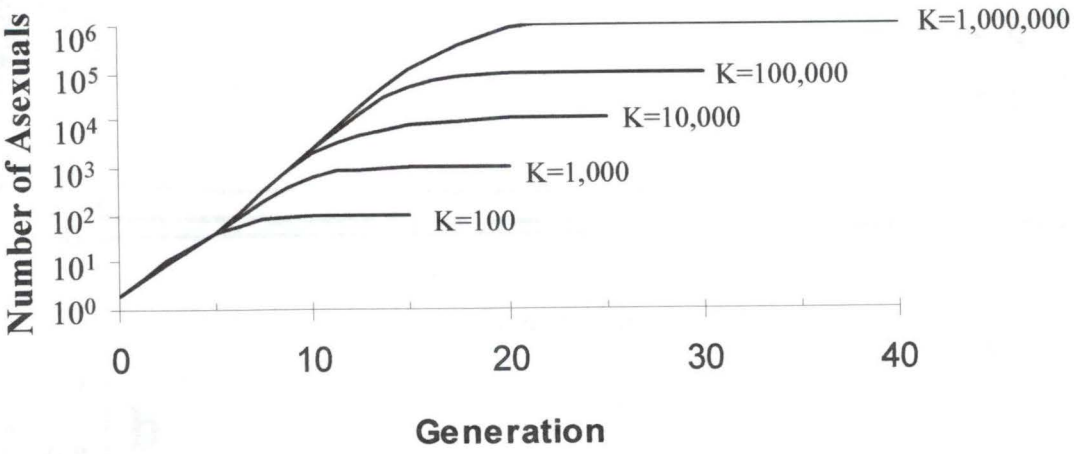
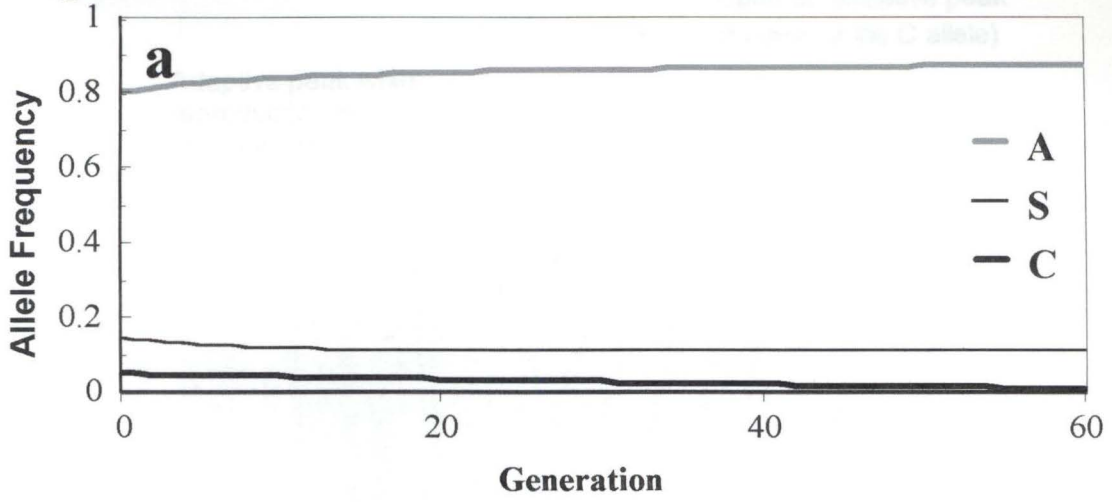
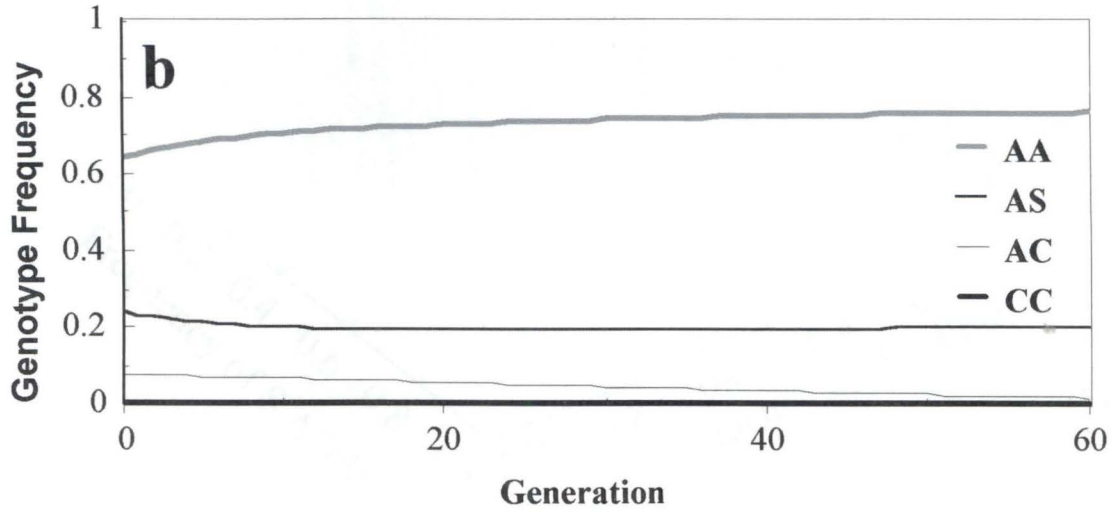

Number of Asexuals
10^6
10^5
10^4
10^3
10^2
10^1
10^0
K=1,000,000
K=100,000
K=10,000
K=1,000
K=100
0
10
20
30
40
Generation

Figure 2.


a
Allele Frequency
1
0.8
0.6
0.4
0.2
0
0
20
40
60
Generation
A
S
C


b
Genotype Frequency
1
0.8
0.6
0.4
0.2
0
0
20
40
60
Generation
AA
AS
AC
CC

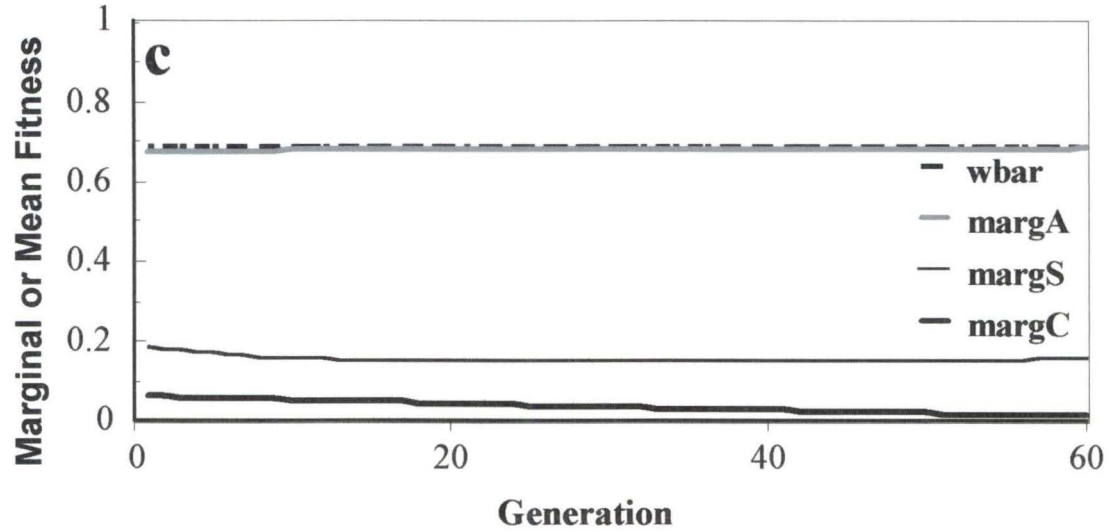

c
Marginal or Mean Fitness
1
0.8
0.6
0.4
0.2
0
0
20
40
60
Generation
wbar
margA
margS
margC

Figure 3.

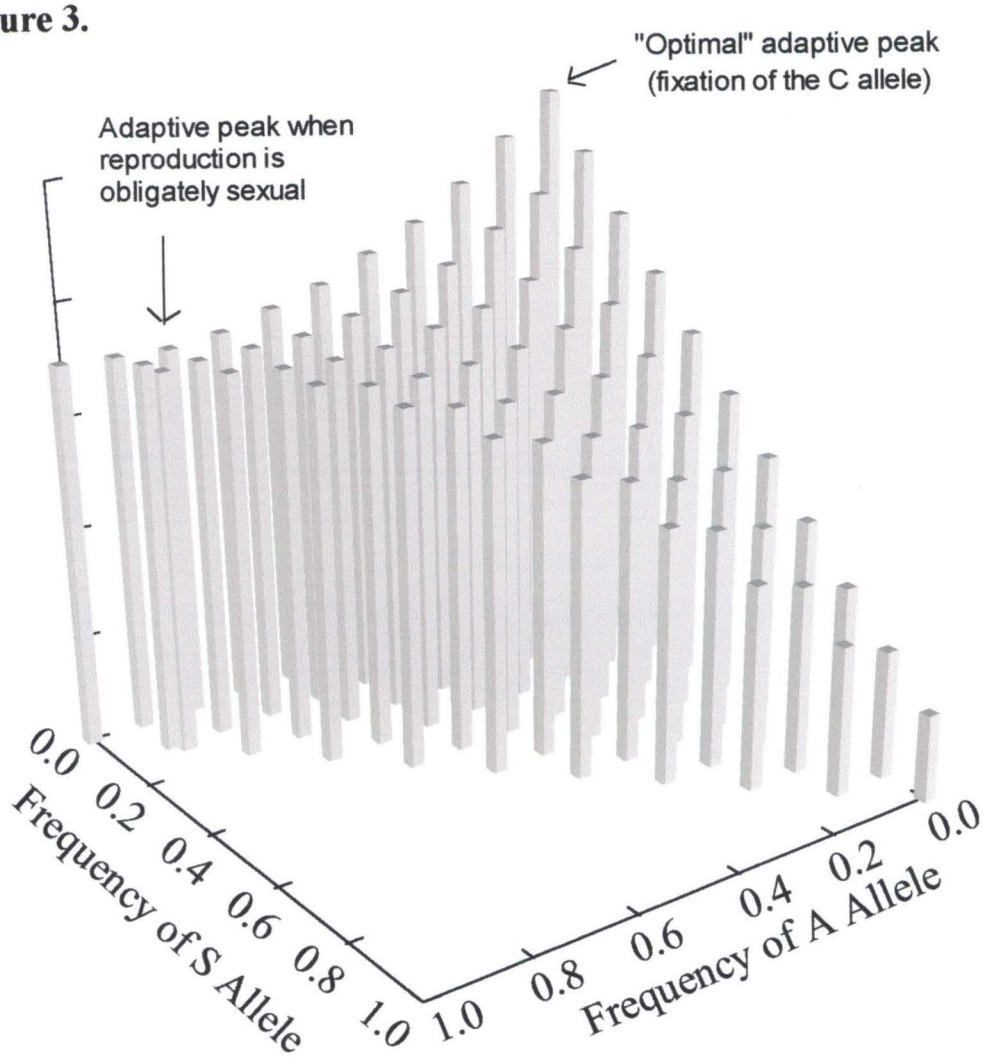

"Optimal" adaptive peak
(fixation of the C allele)
Adaptive peak when
reproduction is
obligately sexual
0.0 0.2 0.4 0.6 0.8 1.0
Frequency of S Allele
1.0 0.8 0.6 0.4 0.2 0.0
Frequency of A Allele

Figure 4.

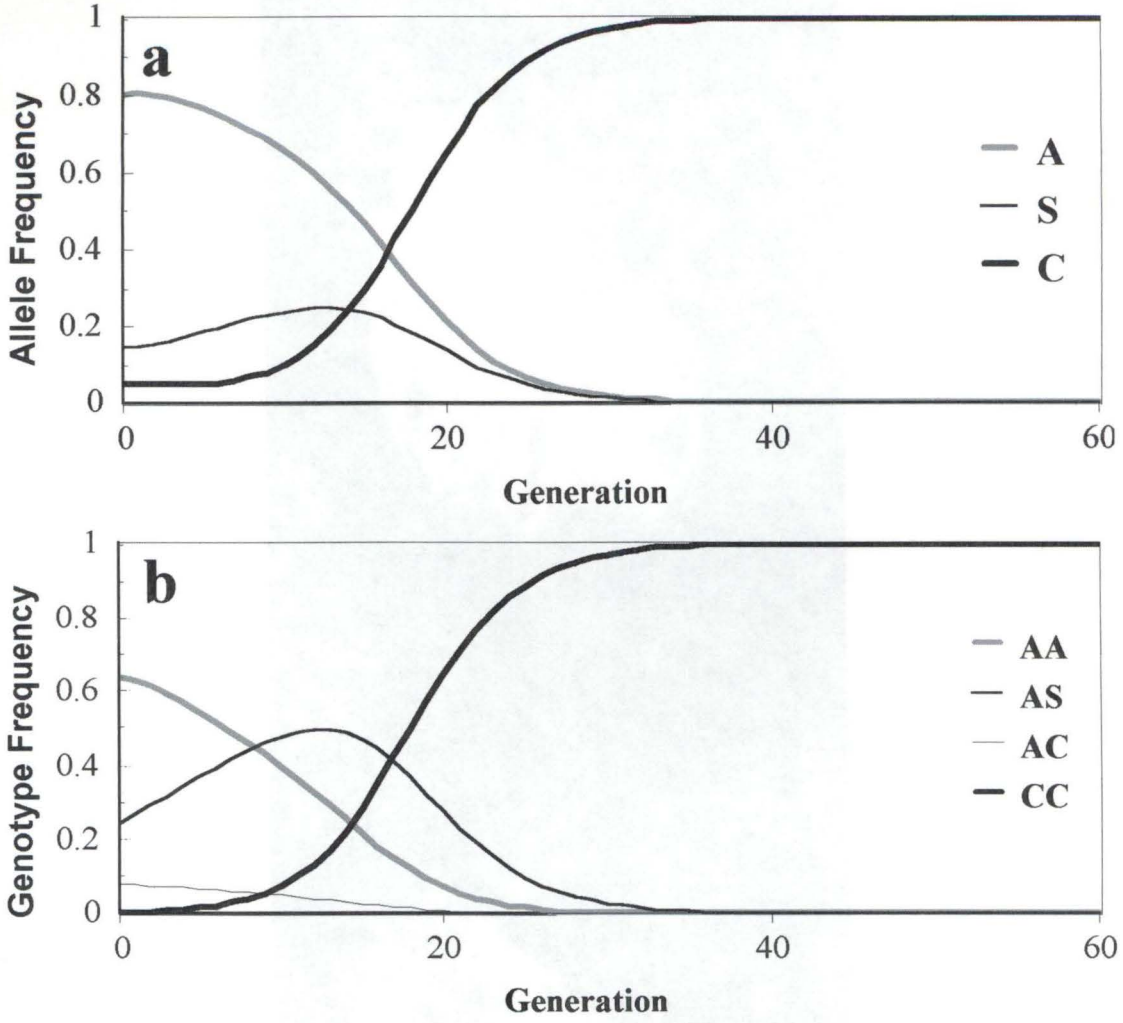

a
Allele Frequency
1
0.8
0.6
0.4
0.2
0
0
20
40
60
Generation
A
S
C
b
Genotype Frequency
1
0.8
0.6
0.4
0.2
0
0
20
40
60
Generation
AA
AS
AC
CC

Figure 5.

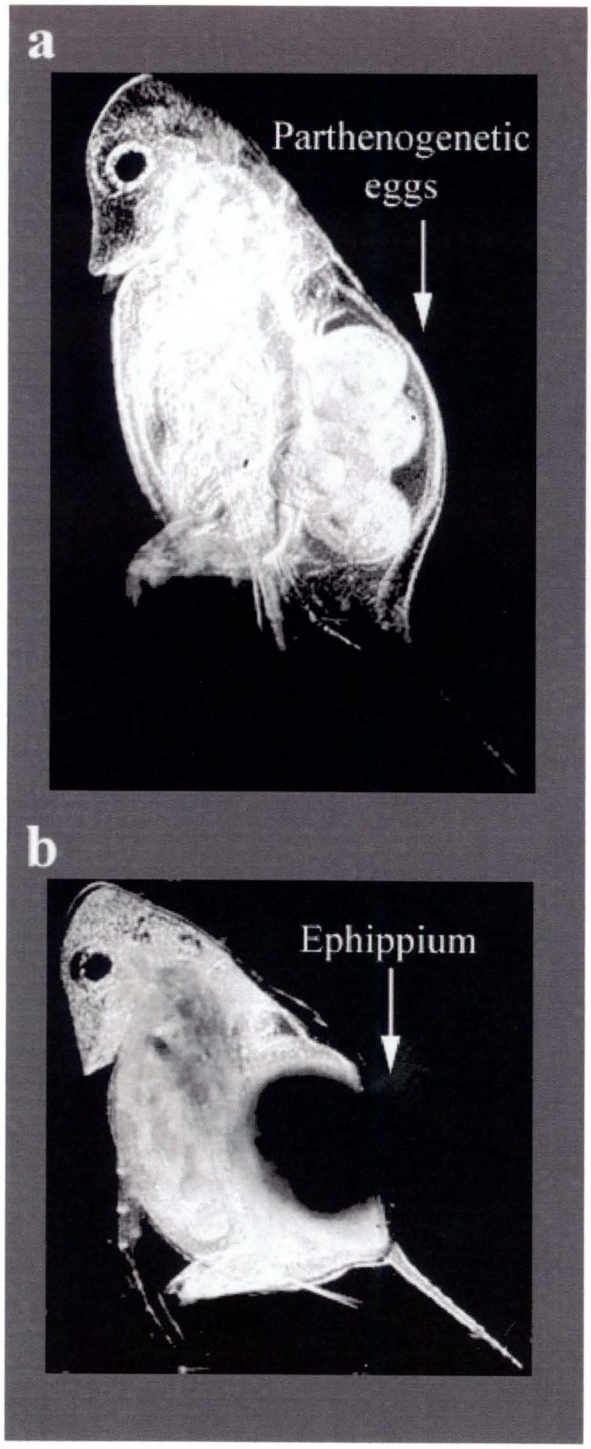
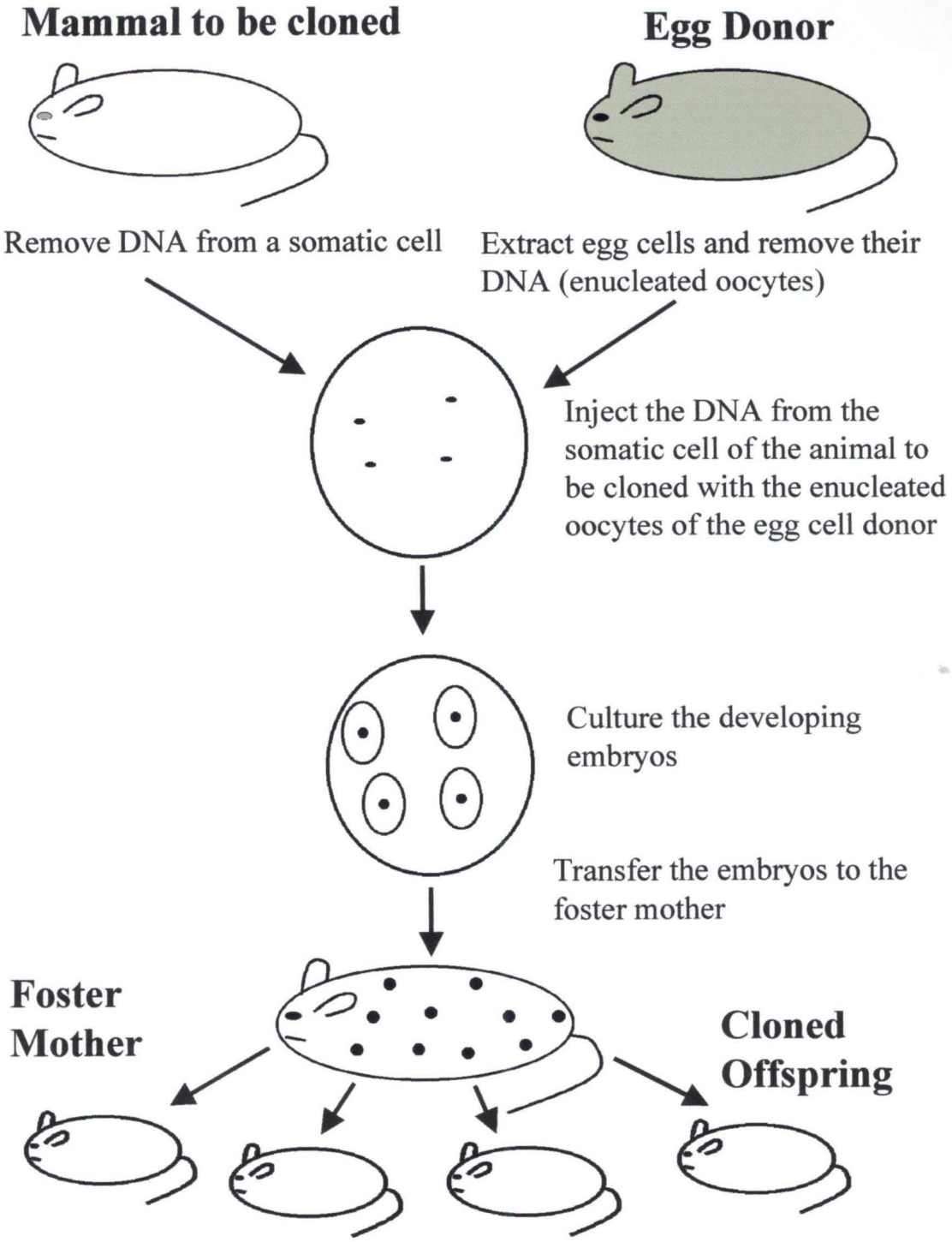


Figure 6.


Mammal to be cloned
Egg Donor
Remove DNA from a somatic cell
Extract egg cells and remove their DNA (enucleated oocytes)
Inject the DNA from the somatic cell of the animal to be cloned with the enucleated oocytes of the egg cell donor
Culture the developing embryos
Transfer the embryos to the foster mother
Foster Mother
Cloned Offspring